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UTILITY PATENT APPLICATION TRANSMITTAL

(Only for new nonprovisional applications under 37 CFR 1.53(b))

Attorney Docket No. B98-031-3

First Named Inventor or Application Identifier Goodman et al.

Title Modulating Robo: Ligand Interactions

EL071086631US

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ADDRESS TO: **Assistant Commissioner for Patents**
Box Patent Application
Washington, D. C. 20231

APPLICATION ELEMENTS

See MPEP chapter 600 concerning utility patent application contents.

1. ____*Fee Transmittal Form
   (Submit an original, and a duplicate for fee processing)

2. _X_ Specification (Total Pages _____)
   (preferred arrangement set forth below)
   - Descriptive Title of the Invention
   - Cross References to Related Applications
   - Statement Regarding Fed sponsored R & D
   - Reference to Microfiche Appendix
   - Background of the Invention
   - Brief Summary of the Invention
   - Brief Description of the Drawings (if filed)
   - Detailed Description
   - Claims
   - Abstract of the Disclosure

3. ____Drawings(s) (35 USC 113) (Total Sheets _____)

4. ____Oath or Declaration (Total Pages _____)
   a. ___ Newly Executed (Original or Copy)
   b. ___ Copy from a Prior Application (37 CFR 1.63(d))
   (for Continuation/Divisional with Box 17 completed)
   i. ___ DELETIONS OF INVENTOR(S) Signed statement attached deleting inventor(s) named in the prior application, see 37 CFR 1.63(d)(2) and 1.33(b).

5. _X_ Incorporation By Reference
   The entire disclosure of the prior application is considered as being part of the disclosure of the accompanying application and is hereby incorporated by reference therein.

6. ____Microfiche Computer Program (Appendix)
7. ____Nucleotide and/or Amino Acid Sequence Submission

(if applicable, all necessary)

a. ____ Computer Readable Copy
b. ____ Paper Copy (identical to computer copy)
c. ____ Statement verifying identity of above copies
d. ____ Request to use CRF from another application

ACCOMPANYING APPLICATION PARTS

8. _____Assignment Papers (cover sheet & documents(s))
_____a. Assignment to_____, of record in prior application
9. ____ 37 CFR 3.73(b) Statement (where there is an assignee)
_____Power of Attorney
10. __ English Translation Document (if applicable)
11. __ a. Information Disclosure Statement (IDS)/PTO-1449
__ b. Copies of IDS Citations
12. _____Preliminary Amendment
13. __X__Return Receipt Postcard (MPEP 503) (Should be specifically itemized)
14. _____a. *Small Entity Statement(s)
_____b. Statement filed in prior application, Status still proper and desired
15. __ Certified Copy of Priority Document(s) (if foreign priority is claimed)
16. __ Other: _____

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17. **If a CONTINUING APPLICATION,** check appropriate box and supply the requisite information below and in a preliminary amendment:

__X__ Continuing __Divisional __Continuation-in-part (CIP) of prior application No: 60/065,544 and 60/081,057

Prior application information: Examiner _____ Group Art Unit _____

18. **Correspondence Address**

_____ Customer Number or Bar Code Label _____
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Modulating Robo:Ligand Interactions

Inventors: Corey S. Goodman, Thomas Kidd, Katja Brose and Marc Tessier-Lavigne

The research carried out in the subject application was supported in part by NIH grant NS18366. The government may have rights in any patent issuing on this application.

CROSS-REFERENCE TO RELATED APPLICATION

This application is a continuing application under 35USC120 of USSN 60/081,057 filed Apr 07, 1998 and of USSN 60/065,544, filed Nov 14, 1997.

INTRODUCTION

Field of the Invention

The field of this invention is methods for modulating nerve cell function.

Background

In the developing CNS, most growth cones confront the midline at one or multiple times during their journey and make the decision of whether to cross or not to cross. This decision is not a static one but rather changes according to the growth cone's history. For example, in the Drosophila ventral nerve cord, about 10% of the interneurons project their axons only on their own side, in some cases extending near the midline without crossing it. The other 90% of the interneurons first project their axons across the midline and then turn to project longitudinally on the other side, often extending near the midline. These growth cones, having crossed the midline once, never cross it again, in spite of their close proximity to the midline and the many commissural axons crossing it. This decision to cross or not to cross is not unique to Drosophila but is common to a variety of midline structures in all bilaterally symmetric nervous systems.

What midline signals and growth cone receptors control whether growth cones do or do not cross the midline? After crossing once, what mechanism prevents these growth cones from crossing again? A related issue concerns the nature of the midline as an intermediate target. If so many growth cones find the midline such an attractive structure, why do they cross over it rather than linger? Why do they leave the midline?

One approach to find the genes encoding the components of such a system is to screen for mutations in which either too many or too few axons cross the midline. Such a large-scale mutant screen was previously conducted in Drosophila, and led to the identification of two key genes: *commissureless* (*comm*) and *roundabout* (*robo*) (Seeger et al., 1993; reviewed by Tear et al., 1993). In *comm* mutant embryos, commissural growth cones initially orient toward the midline but then fail to cross it and instead recoil and extend on their own side. *robo* mutant embryos, on the other hand, display the opposite phenotype in that too many axons cross the midline; many growth cones that normally extend only on their own side instead now project across the midline and axons that normally cross the midline only once instead appear to cross and recross multiple times (Seeger et al, 1993; present disclosure). Double mutants of *comm* and *robo* display a *robo*-like phenotype.

How do *comm* and *robo* function to control midline crossing? Neither the initial paper on these genes (Seeger et al., 1993) nor the cloning of *comm* (Tear et al., 1996) resolved this question. *comm* encodes a novel surface protein expressed on midline cells. In fact, the *comm* paper (Tear et al., 1996) ended with the hope that future work would "... help shed some light on the enigmatic function of Comm."

USSN 08/971,172 (*Robo, A Novel Family of Polypeptides and Nucleic Acids*, by inventors: Corey S. Goodman, Thomas Kidd, Kevin J. Mitchell and Guy Tear) discloses the cloning and characterization of *robo* in various species including Drosophila; Robo polypeptides and polypeptide-encoding nucleic acids are also disclosed and their genbank accession numbers referenced in Kidd et al. (1998) Cell 92, 205-215. *robo* encodes a new class of guidance receptor with 5 immunoglobulin (Ig) domains, 3 fibronectin type III domains, a transmembrane domain, and a long cytoplasmic domain. Robo defines a new subfamily of Ig superfamily proteins that is highly conserved from fruit flies to mammals. The Robo ectodomains, and in particular the first two Ig domains, are highly conserved from fruit fly to human, while the cytoplasmic domains are more divergent. Nevertheless, the cytoplasmic domains contain three highly conserved short proline-rich motifs which may represent binding sites for SH3 or other binding domains in linker or signaling molecules.

For those axons that never cross the midline, Robo is expressed on their growth cones from the outset; for the majority of axons that do cross the midline, Robo is expressed at high levels on their growth cones only after they cross the midline. Transgenic rescue experiments

in Drosophila reveal that Robo can function in a cell autonomous fashion, consistent with it functioning as a receptor. Thus, in Drosophila, Robo appears to function as the gatekeeper controlling midline crossing; growth cones expressing high levels of Robo are prevented from crossing the midline. Robo proteins in mammals function in a similar manner in controlling axon guidance.

USSN 60/065,54 (*Methods for Modulating Nerve Cell Function*, by inventors: Corey S. Goodman, Thomas Kidd, Guy Tear, Claire Russell and Kevin Mitchell) discloses ectopic and overexpression studies revealing that Comm down-regulates Robo expression, demonstrating that Comm functions to suppress the Robo-mediated midline repulsion. These results show that the levels of Comm at the midline and Robo on growth cones are tightly intertwined and dynamically regulated to assure that only certain growth cones cross the midline, that those growth cones that cross do not linger at the midline, and that once they cross they never do so again.

Relevant Literature

Seeger, M., Tear, G., Ferres-Marco, D. and Goodman C.S. (1993) Neuron *10*, 409 - 426; Tear G., et al. (1996) Neuron *16*, 501 - 514; Rothberg et al. (1990) Genes Dev 4, 2169-2187; Kidd et al. (1998) Cell 92, 205-215.

SUMMARY OF THE INVENTION

The invention provides methods and compositions relating to vertebrate Slit1 and Slit2, collectively vertebrate Slit) polypeptides, related nucleic acids, polypeptide domains thereof having vertebrate Slit-specific structure and activity, and modulators of vertebrate Slit function. Vertebrate Slit polypeptides can regulate cell, especially nerve cell, function and morphology. The polypeptides may be produced recombinantly from transformed host cells from the subject vertebrate Slit polypeptide encoding nucleic acids or purified from mammalian cells. The invention provides isolated vertebrate Slit hybridization probes and primers capable of specifically hybridizing with natural vertebrate Slit genes, vertebrate Slit-specific binding agents such as specific antibodies, and methods of making and using the subject compositions in diagnosis (e.g. genetic hybridization screens for vertebrate Slit transcripts), therapy (e.g. to modulate nerve cell growth) and in the biopharmaceutical industry (e.g. as immunogens, reagents for isolating vertebrate Slit genes and polypeptides,

reagents for screening chemical libraries for lead pharmacological agents, etc.).

The invention also provides methods and compositions for identifying agents which modulate the interaction of Robo and a Robo ligand and for modulating the interaction of Robo and a Robo ligand. The methods for identifying Robo:ligand modulators find particular application in commercial drug screens. These methods generally comprise (1) combining a Robo polypeptide, a Slit polypeptide and a candidate agent under conditions whereby, but for the presence of the agent, the Robo and Slit polypeptides engage in a first interaction, and (2) determining a second interaction of the Robo and Slit polypeptides in the presence of the agent, wherein a difference between the first and second interactions indicates that the aget modulates the interaction of the Robo and Slit polypeptides. The subject methods of modulating the interaction of Robo and a Robo ligand involve combining a Robo polypeptide, a Slit polypeptide and a modulator under conditions whereby, but for the presence of the modulator, the Robo and Slit polypeptides engage in a first interaction, whereby the Robo and Slit polypeptides engage in a second interaction different from the first interaction. In a particular embodiment, the modulator is dominant negative form of the Robo or Slit polypeptide.

DETAILED DESCRIPTION OF THE INVENTION

The subject methods include screens for agents which modulate Robo:ligand interactions and methods for modulating Robo:ligand interactions. Robo activation is found to regulate a wide variety of cell functions, including cell-cell interactions, cell mobility, morphology, etc. Slit polypeptides are disclosed as specific activators and inactivators of Robo polypeptides. Accordingly, the invention provides methods for modulating targeted cell function comprising the step of modulating Robo activation by contacting the cell with a modulator of a Robo:Slit interaction..

The targeted Robo polypeptide is generally naturally expressed on the targeted cells. The nucleotide sequences of exemplary natural cDNAs encoding drosophila 1, drosophila 2, C. elegans, human 1, human 2 and mouse 1 Robo polypeptides and their translates are described in Kidd et al. (1998) Cell 92, 205-215 and USSN 08/971,172. The targeted Robo polypeptides comprise at least a functional Robo domain, which domain has Robo-specific amino acid sequence and binding specificity or function. Preferred Robo domains comprise

at least 8, preferably at least 16, more preferably at least 32, most preferably at least 64 consecutive residues of a natural full length Robo. In a particular embodiment, the domains comprise one or more structural/functional Robo immunoglobulin, fibronectin or cytoplasmic motif domains described herein. The subject domains provide Robo-specific antigens and/or immunogens, especially when coupled to carrier proteins. For example, peptides corresponding to Robo- and human Robo-specific domains are covalently coupled to keyhole limpet antigen (KLH) and the conjugate is emulsified in Freunds complete adjuvant. Laboratory rabbits are immunized according to conventional protocol and bled. The presence of Robo-specific antibodies is assayed by solid phase immunosorbant assays using immobilized Robo polypeptides. Generic Robo-specific peptides are readily apparent as conserved regions in aligned Robo polypeptide sequences. In addition, species-specific antigenic and/or immunogenic peptides are readily apparent as diverged extracellular or cytosolic regions in alignments Human Robo-specific antibodies are characterized as uncross-reactive with non-human Robo polypeptides.

The subject domains provide Robo domain specific activity or function, such as Robo-specific cell, especially neuron modulating or modulating inhibitory activity, Robo-ligand-binding or binding inhibitory activity. Robo-specific activity or function may be determined by convenient *in vitro,* cell-based, or *in vivo* assays: e.g. *in vitro* binding assays, cell culture assays, in animals (e.g. gene therapy, transgenics, etc.), etc. The binding target may be a natural intracellular binding target, a Robo regulating protein or other regulator that directly modulates Robo activity or its localization; or non-natural binding target such as a specific immune protein such as an antibody, or a Robo specific agent such as those identified in screening assays such as described below. Robo-binding specificity may be assayed by binding equilibrium constants (usually at least about $10^7 M^{-1}$, preferably at least about $10^8 M^{-1}$, more preferably at least about $10^9 M^{-1}$), by the ability of the subject polypeptide to function as negative mutants in Robo-expressing cells, to elicit Robo specific antibody in a heterologous host (e.g a rodent or rabbit), etc.

Similarly, the Slit polypeptide is conveniently selected from Slit polypeptides which specifically activate or inhibit the activation of the Robo polypeptide. Exemplary suitable Slit polypeptides (a) comprises a vertebrate Slit sequence disclosed herein, especially human Slit-1 (SEQ ID NO:02), or a deletion mutant thereof which specifically modulates Robo

expression or a sequence about 60-70%, preferably about 70-80%, more preferably about 80-90%, more preferably about 90-95%, most preferably about 95-99% similar to a vertebrate Slit sequence disclosed herein as determined by Best Fit analysis using default settings and is other than a natural drosophila Slit sequence, preferably other than a natural invertebrate Slit sequence, and/or (b) is encoded by a nucleic acid comprising a natural Slit encoding sequence (such as a natural human Slit-1 encoding sequence, SEQ ID NO:01) or a fragment thereof at least 36, preferably at least 72, more preferably at least 144, most preferably at least 288 nucleotides in length which specifically hybridizes thereto. Suitable deletion mutants are readily screened in Robo binding or activation assays as described herein. Preferred Slit domains/deletion mutants/fragemtns comprise at least 8, preferably at least 16, more preferably at least 32, most preferably at least 64 consecutive residues of a disclosed vertebrate Slit sequences and provide a Slit specific activity, such as Slit-specific antigenicity and/or immunogenicity, especially when coupled to carrier proteins as described above for Robo above. Suitable natural Slit encoding sequence fragements are of length sufficient to encode such Slit domains. In a particular embodiment, the Slit fragments comprise species specific fragments; such fragments are readily discerned from alignments of the disclosed sequences, see, e.g. shown as white backgrounded sequences in Tables 3 and 4. Exemplary such human Slit-1 immunogenic and/or antigenic peptides are shown in Table 1.

Table 1. Immunogenic human Slit-1 polypeptides eliciting Slit-1 specific rabbit polyclonal antibody: Slit polypeptide-KLH conjugates immunized per protocol described above.

Slit Polypeptide	Immunogenicity	Slit Polypeptide	Immunogenicity
SEQ ID NO:02, res. 1-10	+++	SEQ ID NO:02, res. 561-576	+++
SEQ ID NO:02, res. 29-41	+++	SEQ ID NO:02, res. 683-697	+++
SEQ ID NO:02, res. 75-87	+++	SEQ ID NO:02, res. 768-777	+++
SEQ ID NO:02, res. 92-109	+++	SEQ ID NO:02, res. 798-813	+++
SEQ ID NO:02, res. 132-141	+++	SEQ ID NO:02, res. 882-894	+++
SEQ ID NO:02, res. 192-205	+++	SEQ ID NO:02, res. 934-946	+++
SEQ ID NO:02, res. 258-269	+++	SEQ ID NO:02, res. 1054-1067	+++
SEQ ID NO:02, res. 295-311	+++	SEQ ID NO:02, res. 1181-1192	+++
SEQ ID NO:02, res. 316-330	+++	SEQ ID NO:02, res. 1273-1299	+++
SEQ ID NO:02, res. 373-382	+++	SEQ ID NO:02, res. 1383-1397	+++
SEQ ID NO:02, res. 403-422	+++	SEQ ID NO:02, res. 1468-1477	+++
SEQ ID NO:02, res. 474-485	+++	SEQ ID NO:02, res. 1508-1517	+++

The subject domains provide Slit domain specific activity or function, such as Slit-

specific cell, especially neuron modulating or modulating inhibitory activity, Slit-ligand-binding or binding inhibitory activity. Slit-specific activity or function may be determined by convenient *in vitro,* cell-based, or *in vivo* assays: e.g. *in vitro* binding assays, cell culture assays, in animals (e.g. gene therapy, transgenics, etc.), etc. The binding target may be a natural intracellular binding target, a Slit regulating protein or other regulator that directly modulates Slit activity or its localization; or non-natural binding target such as a specific immune protein such as an antibody, or a Slit specific agent such as those identified in screening assays such as described below. Slit-binding specificity may be assayed by binding equilibrium constants (usually at least about 10^7 M^{-1}, preferably at least about 10^8 M^{-1}, more preferably at least about 10^9 M^{-1}), by the ability of the subject polypeptide to function as negative mutants in Slit-expressing cells, to elicit Slit specific antibody in a heterologous host (e.g a rodent or rabbit), etc.

In one embodiment, the Slit polypeptides are encoded by a nucleic acid comprising SEQ ID NO:01 or a fragment thereof which hybridizes with a full-length strand thereof, preferably under stringent conditions. Such nucleic acids comprise at least 36, preferably at least 72, more preferably at least 144 and most preferably at least 288 nucleotides of SEQ ID NO:01. Demonstrating specific hybridization generally requires stringent conditions, for example, hybridizing in a buffer comprising 30% formamide in 5 x SSPE (0.18 M NaCl, 0.01 M $NaPO_4$, pH7.7, 0.001 M EDTA) buffer at a temperature of 42°C and remaining bound when subject to washing at 42°C with 0.2 x SSPE (Conditions I); preferably hybridizing in a buffer comprising 50% formamide in 5 x SSPE buffer at a temperature of 42°C and remaining bound when subject to washing at 42°C with 0.2 x SSPE buffer at 42°C (Conditions II). Exemplary nucleic acids which hybridize with a strand of SEQ ID NO:01 are shown in Table 2.

Table 2. Exemplary nucleic acids which hybridize with a strand of SEQ ID NO:01 under Conditions I and/or II.

Slit Nucleic Acid	Hybridization	Slit Nucleic Acid	Hybridization
SEQ ID NO:01, nucl. 1-47	+	SEQ ID NO:01, nucl. 1258-1279	+
SEQ ID NO:01, nucl. 58-99	+	SEQ ID NO:01, nucl. 1375-1389	+
SEQ ID NO:01, nucl. 95-138	+	SEQ ID NO:01, nucl. 1581-1595	+
SEQ ID NO:01, nucl. 181-220	+	SEQ ID NO:01, nucl. 1621-1639	+
SEQ ID NO:01, nucl. 261-299	+	SEQ ID NO:01, nucl. 1744-1755	+
SEQ ID NO:01, nucl. 274-315	+	SEQ ID NO:01, nucl. 1951-1969	+
SEQ ID NO:01, nucl. 351-389	+	SEQ ID NO:01, nucl. 2150-2163	+
SEQ ID NO:01, nucl. 450-593	+	SEQ ID NO:01, nucl. 2524-2546	+
SEQ ID NO:01, nucl. 524-546	+	SEQ ID NO:01, nucl. 2761-2780	+
SEQ ID NO:01, nucl. 561-608	+	SEQ ID NO:01, nucl. 2989-2999	+
SEQ ID NO:01, nucl. 689-727	+	SEQ ID NO:01, nucl. 3108-3117	+
SEQ ID NO:01, nucl. 708-737	+	SEQ ID NO:01, nucl. 3338-3351	+
SEQ ID NO:01, nucl. 738-801	+	SEQ ID NO:01, nucl. 3505-3514	+
SEQ ID NO:01, nucl. 805-854	+	SEQ ID NO:01, nucl. 3855-3867	+
SEQ ID NO:01, nucl. 855-907	+	SEQ ID NO:01, nucl. 4010-4025	+
SEQ ID NO:01, nucl. 910-953	+	SEQ ID NO:01, nucl. 4207-4219	+
SEQ ID NO:01, nucl. 1007-1059	+	SEQ ID NO:01, nucl. 4333-4345	+
SEQ ID NO:01, nucl. 1147-1163	+	SEQ ID NO:01, nucl. 4521-4529	+

A wide variety of cell types express Robo polypeptides subject to regulation by the disclosed methods, including many neuronal cells, transformed cells, infected (e.g. virus) cells, etc. Ascertaining Robo binding or activation is readily effected by binding assays or cells function assays as disclosed herein or in the cited copending applications. Accordingly, indications for the subject methods encompass a wide variety of cell types and function, including axon outgrowth, tumor cell invasion or migration, etc. The target cell may reside in culture or in situ, i.e. within the natural host. For in situ applications, the compositions are added to a retained physiological fluid such as blood or synovial fluid. For CNS administration, a variety of techniques are available for promoting transfer of the therapeutic across the blood brain barrier including disruption by surgery or injection, drugs which transiently open adhesion contact between CNS vasculature endothelial cells, and compounds which facilitate translocation through such cells. Slit polypeptides may also be amenable to direct injection or infusion, topical, intratracheal/nasal administration e.g. through aerosol, intraocularly, or within/on implants e.g. fibers e.g. collagen, osmotic pumps, grafts comprising appropriately transformed cells, etc. A particular method of administration involves coating, embedding or derivatizing fibers, such as collagen fibers, protein polymers,

etc. with therapeutic polypeptides. Other useful approaches are described in Otto et al. (1989) J Neuroscience Research 22, 83-91 and Otto and Unsicker (1990) J Neuroscience 10, 1912-1921. Generally, the amount administered will be empirically determined, typically in the range of about 10 to 1000 μg/kg of the recipient and the concentration will generally be in the range of about 50 to 500 μg/ml in the dose administered. Other additives may be included, such as stabilizers, bactericides, etc. will be present in conventional amounts.

In one embodiment, the invention provides administering the subject Slit polypeptides in combination with a pharmaceutically acceptable excipient such as sterile saline or other medium, gelatin, an oil, etc. to form pharmaceutically acceptable compositions. The compositions and/or compounds may be administered alone or in combination with any convenient carrier, diluent, etc. and such administration may be provided in single or multiple dosages. Useful carriers include solid, semi-solid or liquid media including water and non-toxic organic solvents. In another embodiment, the invention provides the subject compounds in the form of a pro-drug, which can be metabolically converted to the subject compound by the recipient host. A wide variety of pro-drug formulations for polypeptide-based therapeutics are known in the art. The compositions may be provided in any convenient form including tablets, capsules, troches, powders, sprays, creams, etc. As such the compositions, in pharmaceutically acceptable dosage units or in bulk, may be incorporated into a wide variety of containers. For example, dosage units may be included in a variety of containers including capsules, pills, etc. The compositions may be advantageously combined and/or used in combination with other therapeutic or prophylactic agents, different from the subject compounds. In many instances, administration in conjunction with the subject compositions enhances the efficacy of such agents, see e.g. *Goodman & Gilman's The Pharmacological Basis of Therapeutics*, 9th Ed., 1996, McGraw-Hill.

In another aspect, the invention provides methods of screening for agents which modulate Robo-Slit interactions. These methods generally involve forming a mixture of a Robo-expressing cell, a Slit polypeptide and a candidate agent, and determining the effect of the agent on the amount of Robo expressed by the cell. The methods are amenable to automated, cost-effective high throughput screening of chemical libraries for lead compounds. Identified reagents find use in the pharmaceutical industries for animal and

human trials; for example, the reagents may be derivatized and rescreened in *in vitro* and *in vivo* assays to optimize activity and minimize toxicity for pharmaceutical development. Cell and animal based neural guidance/repulsion assays are described in detail in the experimental section below.

The amino acid sequences of the disclosed vertebrate Slit polypeptides are used to back-translate Slit polypeptide-encoding nucleic acids optimized for selected expression systems (Holler et al. (1993) Gene 136, 323-328; Martin et al. (1995) Gene 154, 150-166) or used to generate degenerate oligonucleotide primers and probes for use in the isolation of natural Slit-encoding nucleic acid sequences ("GCG" software, Genetics Computer Group, Inc, Madison WI). Slit-encoding nucleic acids used in Slit-expression vectors and incorporated into recombinant host cells, e.g. for expression and screening, transgenic animals, e.g. for functional studies such as the efficacy of candidate drugs for disease associated with Slit-modulated cell function, etc.

The invention also provides nucleic acid hybridization probes and replication / amplification primers having a vertebrate Slit cDNA specific sequence comprising a fragment of a disclosed vertebrate cDNA sequence, and sufficient to effect specific hybridization thereto. Such primers or probes are at least 12, preferably at least 24, more preferably at least 36 and most preferably at least 96 nucleotides in length. Demonstrating specific hybridization generally requires stringent conditions, for example, hybridizing in a buffer comprising 30% formamide in 5 x SSPE (0.18 M NaCl, 0.01 M $NaPO_4$, pH7.7, 0.001 M EDTA) buffer at a temperature of 42°C and remaining bound when subject to washing at 42°C with 0.2 x SSPE; preferably hybridizing in a buffer comprising 50% formamide in 5 x SSPE buffer at a temperature of 42°C and remaining bound when subject to washing at 42°C with 0.2 x SSPE buffer at 42°C. Slit nucleic acids can also be distinguished using alignment algorithms, such as BLASTX (Altschul *et al.* (1990) Basic Local Alignment Search Tool, J Mol Biol 215, 403-410). In addition, the invention provides nucleic acids having a sequence about 60-70%, preferably about 70-80%, more preferably about 80-90%, more preferably about 90-95%, most preferably about 95-99% similar to a vertebrate Slit sequence disclosed herein as determined by Best Fit analysis using default settings and is other than a natural drosophila Slit sequence, preferably other than a natural invertebrate Slit sequence. In a particular embodiment, the Slit polynucleotide fragments comprise species specific

fragments; such fragments are readily discerned from alignments of the disclosed sequences.

The subject nucleic acids are of synthetic/non-natural sequences and/or are recombinant, meaning they comprise a non-natural sequence or a natural sequence joined to nucleotide(s) other than that which it is joined to on a natural chromosome. The subject recombinant nucleic acids comprising the nucleotide sequence of disclosed vertebrate Slit nucleic acids, or fragments thereof, contain such sequence or fragment at a terminus, immediately flanked by (i.e. contiguous with) a sequence other than that which it is joined to on a natural chromosome, or flanked by a native flanking region fewer than 10 kb, preferably fewer than 2 kb, more preferably fewer than 500 bp, which is at a terminus or is immediately flanked by a sequence other than that which it is joined to on a natural chromosome. While the nucleic acids are usually RNA or DNA, it is often advantageous to use nucleic acids comprising other bases or nucleotide analogs to provide modified stability, etc.

The subject nucleic acids find a wide variety of applications including use as translatable transcripts, hybridization probes, PCR primers, diagnostic nucleic acids, etc.; use in detecting the presence of Slit genes and gene transcripts and in detecting or amplifying nucleic acids encoding additional Slit homologs and structural analogs. In diagnosis, Slit hybridization probes find use in identifying wild-type and mutant Slit alleles in clinical and laboratory samples. Mutant alleles are used to generate allele-specific oligonucleotide (ASO) probes for high-throughput clinical diagnoses. In therapy, therapeutic Slit nucleic acids are used to modulate cellular expression or intracellular concentration or availability of active Slit. Exemplary human Slit-1 probes and primers are shown in Table 5 (A and B) and Table 6.

The following examplary assay is offered by way of illustration and not by way of limitation:

EXAMPLES

Protocol for Ligand Screening of Transfected COS cells.

I. Prepare the Ligand

Expression Construct: cDNAs encoding targeted Slit polypeptides are tagged with the Fc portion of human IgG and subcloned into a 293 expression vector (pCEP4: In Vitrogen).

Transfection: 293 EBNA cells are transfected ($CaPO_4$ method) with the Slit

expression constructs. After 24 h recovery, transfected cells are selected with G418 (geneticin, 250 ug/ml, Gibco) and hygromycin (200 ug/ml). Once the selection process is complete, cells are maintained in Dulbecco's Modified Eagles medium (DME)/10% FCS under selection.

Preparation of Conditioned Medium: Serum-containing media is replaced with Optimem with glutamax-1 (Gibco) and 300 ng/ml heparin (Sigma), and the cells are conditioned for 3 days. The media is collected and spun at 3,000xg for 10 minutes. The supernatant is filtered (0.45 um) and stored with 0.1% azide at 4°C for no more than 2 weeks.

II. Prepare Truncated Receptor (Positive Control)

Expression Construct: cDNA encoding a corresponding Robo C-terminal deletion mutant comprising the extracellular domain (truncated immediately N-terminal to the transmembrane region) is subcloned into a 293 expression vector (pCEP4: In Vitrogen).

Transfection: 293 EBNA cells are transfected ($CaPO_4$ method) with the receptor mutant expression construct. After 24 h recovery, transfected cells are selected with G418 (geneticin, 250 ug/ml, Gibco) and hygromycin (200 ug/ml). Once the selection process is complete, cells are maintained in Dulbecco's Modified Eagles medium (DME)/10% FCS under selection.

Preparation of Conditioned Medium: Serum-containing media is replaced with Optimem with glutamax-1 (Gibco) and 300 ng/ml heparin (Sigma), and the cells are conditioned for 3 days. The media is collected and spun at 3,000xg for 10 minutes. The supernatant is filtered (0.45 um) and stored with 0.1% azide at 4°C for no more than 2 weeks.

II. Transfect COS Cells

Seed COS cells (250,000) on 35 mm dishes in 2 ml DME/10% FCS.

18-24 h later, dilute 1 ug of Robo-encoding DNA (cDNA cloned into pMT21 expression vector) into 200 ul serum-free media and add 6 ul of Lipofectamine (Gibco). Incubate this solution at room temperature for 15-45 min.

Wash the cells 2X with PBS. Add 800 ul serum-free media to the tube containing the lipid-DNA complexes. Overlay this solution onto the washed cells.

Incubate for 6 h. Stop the reaction by adding 1 ml DMA/20% FCS. Refeed cells. Assay cells 12 hr later.

III. Ligand Binding Assay

Wash plates of transfected COS cells 1X with cold PBS (plus Ca/Mg)/1% goat serum. Add 1 ml conditioned media neat and incubate 90 min at room temp.

Wash plates 3X with PBS (plus Ca/Mg). On the 4th wash, add 1 ml 50% methanol to 1 ml PBS. Then add 1 ml methanol. Evacuate and add 1 ml methanol.

Wash 1X with PBS. Wash 1X PBS/1% goat serum.

Add secondary antibody (1-to-2,000 anti-human Fc conjugated to alkaline phosphatase (Jackson Lab)) in PBS/1% goat serum. Incubate 30-40 min room temp.

Wash 3X with PBS. Wash 1X alkaline phosphatase buffer (100 mM Tris-Cl, pH 9.5, 100 mM NaCl, 5 mM $MgCl_2$). Prepare alkaline phosphatase reagents: 4.5 ul/ml NBT and 3.5 ul/ml BCIP (Gibco) in alkaline phosphatase buffer.

Incubate 10-30 min, quench with 20 mM EDTA in PBS. Cells that have bound Slit polypeptides are visible by the presence of a dark purple reaction product.

In parallel incubations, positive controls are provided by titrating Slit binding with serial dilutions of the mutant receptor conditioned medium.

IV. Results: Binding of Slit to Robo

Cell expressing mammalian Slit polypeptides were shown to bind Robo. No reactivity was observed with control COS cells or with receptor-expressing COS cells in the presence of the secondary antibody but in the absence of the Slit-Fc fusion. Binding was observed to receptor-expression cells using a construct in which a Slit polypeptide is fused directly to alkaline phosphatase, for which a secondary antibody is not required. Receptor deletion mutants titrate the Slit-Robo binding, serving as a positive control for inhibition assays.

<u>Protocol for high throughput Robo-Slit binding assay.</u>

A. Reagents:

- <u>Neutralite Avidin</u>: 20 μg/ml in PBS.

- <u>Blocking buffer</u>: 5% BSA, 0.5% Tween 20 in PBS; 1 hour at room temperature.

- <u>Assay Buffer</u>: 100 mM KCl, 20 mM HEPES pH 7.6, 1 mM $MgCl_2$, 1% glycerol, 0.5% NP-40, 50 mM β-mercaptoethanol, 1 mg/ml BSA, cocktail of protease inhibitors.

- <u>^{33}P Robo polypeptide 10x stock</u>: 10^{-8} - 10^{-6} M "cold" Robo polypeptide specific Robo domain supplemented with 200,000-250,000 cpm of labeled Robo (Beckman counter). Place in the 4°C microfridge during screening.

- Protease inhibitor cocktail (1000X): 10 mg Trypsin Inhibitor (BMB # 109894), 10 mg Aprotinin (BMB # 236624), 25 mg Benzamidine (Sigma # B-6506), 25 mg Leupeptin (BMB # 1017128), 10 mg APMSF (BMB # 917575), and 2mM $NaVO_3$ (Sigma # S-6508) in 10 ml of PBS.

-Slit: 10^{-7} - 10^{-5} M biotinylated Slit in PBS.

B. Preparation of assay plates:

- Coat with 120 µl of stock N-Avidin per well overnight at 4°C.
- Wash 2 times with 200 µl PBS.
- Block with 150 µl of blocking buffer.
- Wash 2 times with 200 µl PBS.

C. Assay:

- Add 40 µl assay buffer/well.
- Add 10 µl compound or extract.
- Add 10 µl ^{33}P-Robo (20-25,000 cpm/0.1-10 pmoles/well =10^{-9}- 10^{-7} M final conc).
- Shake at 25°C for 15 minutes.
- Incubate additional 45 minutes at 25°C.
- Add 40 µM biotinylated Slit (0.1-10 pmoles/40 ul in assay buffer)
- Incubate 1 hour at room temperature.
- Stop the reaction by washing 4 times with 200 µM PBS.
- Add 150 µM scintillation cocktail.
- Count in Topcount.

D. Controls for all assays (located on each plate):

a. Non-specific binding

b. Soluble (non-biotinylated Slit) at 80% inhibition.

All publications and patent applications cited in this specification are herein incorporated by reference as if each individual publication or patent application were specifically and individually indicated to be incorporated by reference. Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, it will be readily apparent to those of ordinary skill in the art in light of the teachings of this invention that certain changes and modifications may be made thereto without departing from the spirit or scope of the appended claims.

WHAT IS CLAIMED IS:

1. A method of identifying agents which modulate the interaction of Robo and a Robo ligand, said method comprising the steps of:

combining a Robo polypeptide, a Slit polypeptide and a candidate agent under conditions whereby, but for the presence of the agent, the Robo and Slit polypeptides engage in a first interaction, wherein the Slit polypeptide specifically binds, activates or inhibits the activation of the Robo polypeptide and

determining a second interaction of the Robo and Slit polypeptides in the presence of the agent,

wherein a difference between the first and second interactions indicates that the aget modulates the interaction of the Robo and Slit polypeptides.

2. A method of modulating the interaction of Robo and a Robo ligand, said method comprising the step of

combining a Robo polypeptide, a Slit polypeptide and a modulator under conditions whereby, but for the presence of the modulator, the Robo and Slit polypeptides engage in a first interaction, wherein the Slit polypeptide specifically binds, activates or inhibits the activation of the Robo polypeptide and

whereby the Robo and Slit polypeptides engage in a second interaction different from the first interaction.

3. A method according to claim 2, wherein the modulator is a dominant negative form of the Robo or Slit polypeptide.

4. An isolated Slit polypeptide comprising a vertebrate species-specific Slit fragment.

5. An isolated vertebrate Slit polypeptide according to claim 4, wherein said vertebrate is human, mouse or rat.

6. A recombinant nucleic acid encoding a vertebrate Slit polypeptide according to claim 4.

7. A recombinant Slit nucleic acid comprising a strand of SEQ ID NO:01, or a fragment thereof having at least 24 consecutive nucleotides thereof, and sufficient to specifically hybridize with a polynucleotide having the sequence defined by the corresponding opposite strand of SEQ ID NO:01, and is other than a natural drosophila Slit sequence.

ABSTRACT OF THE DISCLOSURE

Disclosed are methods and compositions for identifying agents which modulate the interaction of Robo and a Robo ligand and for modulating the interaction of Robo and a Robo ligand. The methods for identifying Robo:ligand modulators find particular application in commercial drug screens. These methods generally comprise (1) combining a Robo polypeptide, a Slit polypeptide and a candidate agent under conditions whereby, but for the presence of the agent, the Robo and Slit polypeptides engage in a first interaction, and (2) determining a second interaction of the Robo and Slit polypeptides in the presence of the agent, wherein a difference between the first and second interactions indicates that the aget modulates the interaction of the Robo and Slit polypeptides. The subject methods of modulating the interaction of Robo and a Robo ligand involve combining a Robo polypeptide, a Slit polypeptide and a modulator under conditions whereby, but for the presence of the modulator, the Robo and Slit polypeptides engage in a first interaction, whereby the Robo and Slit polypeptides engage in a second interaction different from the first interaction. In a particular embodiment, the modulator is dominant negative form of the Robo or Slit polypeptide.

SEQ ID NO: 12

SEQ ID NO: 1

Sequence of Human Slit-1 SEQ ID NO: 2

DNA sequence and predicted protein product. Base pair and amino acid number are indicated on the right hand side.

```
ATGCGCGGCGTTGGCTGGCAGATGCTGTCCCTGTCGCTGGGGTTAGTGCTGGCGATCCTGAACAAGGTGGCACCG    75
 M  R  G  V  G  W  Q  M  L  S  L  S  L  G  L  V  L  A  I  L  N  K  V  A  P    25

CAGGCGTGCCCGGCGCAGTGCTCTTGCTCGGGCAGCACAGTGGACTGTCACGGGCTGGCGCTGCGCAGCGTGCCC   150
 Q  A  C  P  A  Q  C  S  C  S  G  S  T  V  D  C  H  G  L  A  L  R  S  V  P    50

AGGAATATCCCCCGCAACACCGAGAGACTGGATTTAAATGGAAATAACATCACAAGAATTACGAAGACAGATTTT   225
 R  N  I  P  R  N  T  E  R  L  D  L  N  G  N  N  I  T  R  I  T  K  T  D  F    75

GCTGGTCTTAGACATCTAAGAGTTCTTCAGCTTATGGAGAATAAGATTAGCACCATTGAAAGAGGAGCATTCCAG   300
 A  G  L  R  H  L  R  V  L  Q  L  M  E  N  K  I  S  T  I  E  R  G  A  F  Q    100

GATCTTAAAGAACTAGAGAGACTGCGTTTAAACAGAAATCACCTTCAGCTGTTTCCTGAGTTGCTGTTTCTTGGG   375
 D  L  K  E  L  E  R  L  R  L  N  R  N  H  L  Q  L  F  P  E  L  L  F  L  G    125

ACTGCGAAGCTATACAGGCTTGATCTCAGTGAAAACCAAATTCAGGCAATCCCAAGGAAAGCTTTCCGTGGGGCA   450
 T  A  K  L  Y  R  L  D  L  S  E  N  Q  I  Q  A  I  P  R  K  A  F  R  G  A    150

GTTGACATAAAAAATTTGCAACTGGATTACAACCAGATCAGCTGTATTGAAGATGGGGCATTCAGGGCTCTCCGG   525
 V  D  I  K  N  L  Q  L  D  Y  N  Q  I  S  C  I  E  D  G  A  F  R  A  L  R    175

GACCTGGAAGTGCTCACTCTCAACAATAACAACATTACTAGACTTTCTGTGGCAAGTTTCAACCATATGCCTAAA   600
 D  L  E  V  L  T  L  N  N  N  N  I  T  R  L  S  V  A  S  F  N  H  M  P  K    200

CTTAGGACTTTTCGACTGCATTCAAACAACCTGTATTGTGACTGCCACCTGGCCTGGCTCTCCGACTGGCTTCGC   675
 L  R  T  F  R  L  H  S  N  N  L  Y  C  D  C  H  L  A  W  L  S  D  W  L  R    225

AAAAGGCCTCGGGTTGGTCTGTACACTCAGTGTATGGGCCCCTCCCACCTGAGAGGCCATAATGTAGCCGAGGTT   750
 K  R  P  R  V  G  L  Y  T  Q  C  M  G  P  S  H  L  R  G  H  N  V  A  E  V    250

CAAAAACGAGAATTTGTCTGCAGTGATGAGGAAGAAGGTCACCAGTCATTTATGGCTCCTTCTTGTAGTGTTTTG   825
 Q  K  R  E  F  V  C  S  D  E  E  E  G  H  Q  S  F  M  A  P  S  C  S  V  L    275

CACTGCCCTGCCGCCTGTACCTGTAGCAACAATATCGTAGACTGTCGTGGGAAAGGTCTCACTGAGATCCCCACA   900
 H  C  P  A  A  C  T  C  S  N  N  I  V  D  C  R  G  K  G  L  T  E  I  P  T    300

AATCTTCCAGAGACCATCACAGAAATACGTTTGGAACAGAACACAATCAAAGTCATCCCTCCTGGAGCTTTCTCA   975
 N  L  P  E  T  I  T  E  I  R  L  E  Q  N  T  I  K  V  I  P  P  G  A  F  S    325

CCATATAAAAAGCTTAGACGAATTGACCTGAGCAATAATCAGATCTCTGAACTTGCACCAGATGCTTTCCAAGGA  1050
 P  Y  K  K  L  R  R  I  D  L  S  N  N  Q  I  S  E  L  A  P  D  A  F  Q  G    350

CTACGCTCTCTGAATTCACTTGTCCTCTATGGAAATAAAATCACAGAACTCCCCAAAAGTTTATTTGAAGGACTG  1125
 L  R  S  L  N  S  L  V  L  Y  G  N  K  I  T  E  L  P  K  S  L  F  E  G  L    375

TTTTCCTTACAGCTCCTATTATTGAATGCCAACAAGATAAACTGCCTTCGGGTAGATGCTTTTCAGGATCTCCAC  1200
 F  S  L  Q  L  L  L  L  N  A  N  K  I  N  C  L  R  V  D  A  F  Q  D  L  H    400

AACTTGAACCTTCTCTCCCTATATGACAACAAGCTTCAGACCATCGCCAAGGGGACCTTTTCACCTCTTCGGGCC  1275
 N  L  N  L  L  S  L  Y  D  N  K  L  Q  T  I  A  K  G  T  F  S  P  L  R  A    425

ATTCAAACTATGCATTTGGCCCAGAACCCCTTTATTTGTGACTGCCATCTCAAGTGGCTAGCGGATTATCTCCAT  1350
 I  Q  T  M  H  L  A  Q  N  P  F  I  C  D  C  H  L  K  W  L  A  D  Y  L  H    450

ACCAACCCGATTGAGACCAGTGGTGCCCGTTGCACCAGCCCCCGCCGCCTGGCAAACAAAAGAATTGGACAGATC  1425
 T  N  P  I  E  T  S  G  A  R  C  T  S  P  R  R  L  A  N  K  R  I  G  Q  I    475

AAAAGCAAGAAATTCCGTTGTTCAGGTACAGAAGATTATCGATCAAAATTAAGTGGAGACTGCTTTGCGGATCTG  1500
 K  S  K  K  F  R  C  S  G  T  E  D  Y  R  S  K  L  S  G  D  C  F  A  D  L    500
```

GCTTGCCCTGAAAAGTGTCGCTGTGAAGGAACCACAGTAGATTGCTCTAATCAAAAGCTCAACAAAATCCCGGAG 1575
A C P E K C R C E G T T V D C S N Q K L N K I P E 525

CACATTCCCCAGTACACTGCAGAGTTGCGTCTCAATAATAATGAATTTACCGTGTTGGAAGCCACAGGAATCTTT 1650
H I P Q Y T A E L R L N N N E F T V L E A T G I F 550

AAGAAACTTCCTCAATTACGTAAAATAAACTTTAGCAACAATAAGATCACAGATATTGAGGAGGGAGCATTTGAA 1725
K K L P Q L R K I N F S N N K I T D I E E G A F E 575

GGAGCATCTGGTGTAAATGAAATACTTCTTACGAGTAATCGTTTGGAAAATGTGCAGCATAAGATGTTCAAGGGA 1800
G A S G V N E I L L T S N R L E N V Q H K M F K G 600

TTGGAAAGCCTCAAAACTTTGATGTTGAGAAGCAATCGAATAACCTGTGTGGGGAATGACAGTTTCATAGGACTC 1875
L E S L K T L M L R S N R I T C V G N D S F I G L 625

AGTTCTGTGCGTTTGCTTTCTTTGTATGATAATCAAATTACTACAGTTGCACCAGGGGCATTTGATACTCTCCAT 1950
S S V R L L S L Y D N Q I T T V A P G A F D T L H 650

TCTTTATCTACTCTAAACCTCTTGGCCAATCCTTTTAACTGTAACTGCTACCTGGCTTGGTTGGGAGAGTGGCTG 2025
S L S T L N L L A N P F N C N C Y L A W L G E W L 675

AGAAAGAAGAGAATTGTCACGGGAAATCCTAGATGTCAAAAACCATACTTCCTGAAAGAAATACCCATCCAGGAT 2100
R K K R I V T G N P R C Q K P Y F L K E I P I Q D 700

GTGGCCATTCAGGACTTCACTTGTGATGACGGAAATGATGACAATAGTTGCTCCCCACTTTCTCGCTGTCCTACT 2175
V A I Q D F T C D D G N D D N S C S P L S R C P T 725

GAATGTACTTGCTTGGATACAGTCGTCCGATGTAGCAACAAGGGTTTGAAGGTCTTGCCGAAAGGTATTCCAAGA 2250
E C T C L D T V V R C S N K G L K V L P K G I P R 750

GATGTCACAGAGTTGTATCTGGATGGAAACCAATTTACACTGGTTCCCAAGGAACTCTCCAACTACAAACATTTA 2325
D V T E L Y L D G N Q F T L V P K E L S N Y K H L 775

ACACTTATAGACTTAAGTAACAACAGAATAAGCACGCTTTCTAATCAGAGCTTCAGCAACATGACCCAGCTCCTC 2400
T L I D L S N N R I S T L S N Q S F S N M T Q L L 800

ACCTTAATTCTTAGTTACAACCGTCTGAGATGTATTCCTCCTCGCACCTTTGATGGATTAAAGTCTCTTCGATTA 2475
T L I L S Y N R L R C I P P R T F D G L K S L R L 825

CTTTCTCTACATGGAAATGACATTTCTGTTGTGCCTGAAGGTGCTTTCAATGATCTTTCTGCATTATCACATCTA 2550
L S L H G N D I S V V P E G A F N D L S A L S H L 850

GCAATTGGAGCCAACCCTCTTTACTGTGATTGTAACATGCAGTGGTTATCCGACTGGGTGAAGTCGGAATATAAG 2625
A I G A N P L Y C D C N M Q W L S D W V K S E Y K 875

GAGCCTGGAATTGCTCGTTGTGCTGGTCCTGGAGAAATGGCAGATAAACTTTTACTCACAACTCCCTCCAAAAAA 2700
E P G I A R C A G P G E M A D K L L L T T P S K K 900

TTTACCTGTCAAGGTCCTGTGGATGTCAATATTCTAGCTAAGTGTAACCCCTGCCTATCAAATCCGTGTAAAAAT 2775
F T C Q G P V D V N I L A K C N P C L S N P C K N 925

GATGGCACATGTAATAGTGATCCAGTTGACTTTTACCGATGCACCTGTCCATATGGTTTCAAGGGGCAGGACTGT 2850
D G T C N S D P V D F Y R C T C P Y G F K G Q D C 950

GATGTCCCAATTCATGCCTGCATCAGTAACCCATGTAAACATGGAGGAACTTGCCACTTAAAGGAAGGAGAAGAA 2925
D V P I H A C I S N P C K H G G T C H L K E G E E 975

GATGGATTCTGGTGTATTTGTGCTGATGGATTTGAAGGAGAAAATTGTGAAGTCAACGTTGATGATTGTGAAGAT 3000
D G F W C I C A D G F E G E N C E V N V D D C E D 1000

AATGACTGTGAAAATAATTCTACATGTGTCGATGGCATTAATAACTACACATGCCTTTGCCCACCTGAGTATACA 3075
N D C E N N S T C V D G I N N Y T C L C P P E Y T 1025

GGTGAGTTGTGTGAGGAGAAGCTGGACTTCTGTGCCCAGGACCTGAACCCCTGCCAGCACGATTCAAAGTGCATC 3150

G E L C E E K L D F C A Q D L N P C Q H D S K C I 1050

CTAACTCCAAAGGGATTCAAATGTGACTGCACACCAGGGTACGTAGGTGAACACTGCGACATCGATTTTGACGAC 3225

L T P K G F K C D C T P G Y V G E H C D I D F D D 1075

TGCCAAGACAACAAGTGTAAAAACGGAGCCCACTGCACAGATGCAGTGAACGGCTATACGTGCATATGCCCCGAA 3300

C Q D N K C K N G A H C T D A V N G Y T C I C P E 1100

GGTTACAGTGGCTTGTTCTGTGAGTTTTCTCCACCCATGGTCCTCCCTCGTACCAGCCCCTGTGATAATTTTGAT 3375

G Y S G L F C E F S P P M V L P R T S P C D N F D 1125

TGTCAGAATGGAGCTCAGTGTATCGTCAGAATAAATGAGCCAATATGTCAGTGTTTGCCTGGCTATCAGGGAGAA 3450

C Q N G A Q C I V R I N E P I C Q C L P G Y Q G E 1150

AAGTGTGAAAAATTGGTTAGTGTGAATTTTATAAACAAAGAGTCTTATCTTCAGATTCCTTCAGCCAAGGTTCGG 2525

K C E K L V S V N F I N K E S Y L Q I P S A K V R 1175

CCTCAGACGAACATAACACTTCAGATTGCCACAGATGAAGACAGCGGAATCCTCCTGTATAAGGGTGACAAAGAC 3600

P Q T N I T L Q I A T D E D S G I L L Y K G D K D 1200

CATATCGCGGTAGAACTCTATCGGGGGCGTGTTCGTGCCAGCTATGACACCGGCTCTCATCCAGCTTCTGCCATT 3675

H I A V E L Y R G R V R A S Y D T G S H P A S A I 1225

TACAGTGTGGAGACAATCAATGATGGAAACTTCCACATTGTGGAACTACTTGCCTTGGATCAGAGTCTCTCTTTG 3750

Y S V E T I N D G N F H I V E L L A L D Q S L S L 1250

TCCGTGGATGGTGGGAACCCCAAAATCATCACTAACTTGTCAAAGCAGTCCACTCTGAATTTTGACTCTCCACTC 3825

S V D G G N P K I I T N L S K Q S T L N F D S P L 1275

TATGTAGGAGGCATGCCAGGGAAGAGTAACGTGGCATCTCTGCGCCAGGCCCCTGGGCAGAACGGAACCAGCTTC 3900

Y V G G M P G K S N V A S L R Q A P G Q N G T S F 1300

CACGGCTGCATCCGGAACCTTTACATCAACAGTGAGCTGCAGGACTTCCAGAAGGTGCCGATGCAAACAGGCATT 3975

H G C I R N L Y I N S E L Q D F Q K V P M Q T G I 1325

TTGCCTGGCTGTGAGCCATGCCACAAGAAGGTGTGTGCCCATGGCACATGCCAGCCCAGCAGCCAGGCAGGCTTC 4050

L P G C E P C H K K V C A H G T C Q P S S Q A G F 1350

ACCTGCGAGTGCCAGGAAGGATGGATGGGGCCCCTCTGTGACCAACGGACCAATGACCCTTGCCTTGGAAATAAA 4125

T C E C Q E G W M G P L C D Q R T N D P C L G N K 1375

TGCGTACATGGCACCTGCTTGCCCATCAATGCGTTCTCCTACAGCTGTAAGTGCTTGGAGGGCCATGGAGGTGTC 4200

C V H G T C L P I N A F S Y S C K C L E G H G G V 1400

CTCTGTGATGAAGAGGAGGATCTGTTTAACCCATGCCAGGCGATCAAGTGCAAGCATGGGAAGTGCAGGCTTTCA 4275

L C D E E E D L F N P C Q A I K C K H G K C R L S 1425

GGTCTGGGGCAGCCCTACTGTGAATGCAGCAGTGGATACACGGGGGACAGCTGTGATCGAGAAATCTCTTGTCGA 4350

G L G Q P Y C E C S S G Y T G D S C D R E I S C R 1450

GGGGAAAGGATAAGAGATTATTACCAAAAGCAGCAGGGCTATGCTGCTTGCCAAACAACCAAGAAGGTGTCCCGA 4425

G E R I R D Y Y Q K Q Q G Y A A C Q T T K K V S R 1475

TTAGAGTGCAGAGGTGGGTGTGCAGGAGGGCAGTGCTGTGGACCGCTGAGGAGCAAGCGGCGGAAATACTCTTTC 4500

L E C R G G C A G G Q C C G P L R S K R R K Y S F 1500

GAATGCACTGACGGCTCCTCCTTTGTGGACGAGGTTGAGAAAGTGGTGAAGTGCGGCTGTACGAGGTGTGTGTCC 4575

E C T D G S S F V D E V E K V V K C G C T R C V S 1525

Features of Human Slit-1 predicted protein
Co-ordinates refer to amino acid number.

Signal sequence:	7-24	
First amino-flanking sequence:	28-59	
First set of Leucine Rich Repeats:	60-179	(6 repeats)
First carboxy-flanking sequence:	180-276	
Second amino-flanking sequence:	277-308	
Second set of Leucine Rich Repeats:	309-434	(5 repeats)
Second carboxy-flanking sequence:	435-501	
Third amino-flanking sequence:	502-533	
Third set of Leucine Rich Repeats:	534-660	(5 repeats)
Third carboxy-flanking sequence:	661-722	
Fourth amino-flanking sequence:	723-754	
Fourth set of Leucine Rich Repeats:	755-855	(4 repeats)
Fourth carboxy-flanking sequence:	856-917	
First EGF repeat:	918-952	
Second EGF repeat:	953-993	
Third EGF repeat:	994-1031	
Fourth EGF repeat:	1032-1071	
Fifth EGF repeat:	1072-1109	
Spacer:	1110-1116	
Sixth EGF repeat:	1117-1154	
"99aa spacer":	1155-1329	
Seventh EGF repeat:	1330-1366	
Eighth EGF repeat:	1367-1404	
Nineth EGF repeat:	1405-1447	
Cysteine knot motif:	1448-1525	

Leucine rich repeats (LRRs) are predicted by comparison with known proteins and by the presence of the core sequence: xxxFxxLxxLxxLxLxxNxIxxL, where x is any amino acid. In slit proteins, the LRRs are flanked by conserved sequences referred to as the amino- and carboxy- flanking regions. These flanking regions are found in other known proteins, but only in a few instances are both the amino- and carboxy- flank regions present in a single protein. The amino flank region is defined by the consensus: CPxxCxC[1-6x]GxxVDCxxxGL[2-4x]αPxxαPxdttx where x is any amino acid, [x] represents a variable number of amino acids and α is a hydrophobic residue. Lower case indicates a residue is not highly conserved at a particular position. The carboxy flank region is defined by the consensus: PßxCγCxα[1-5x]Wα[14-26x]RCxxPxxxxxxxαxxαxxxxF[1-3x]Cs[3-17x] where ß is W or a hydrophobic residue, γ is D or N and α is a hydrophobic residue.
Epidermal growth factor (EGF) repeats are predicted by the consensus: CxxxxCxngxC[6-9x]αxCxCxxGαxGxxCxxxxxx.

The so called "99aa spacer" is actually ~200 amino acids in the Drosophila protein and 174 amino acids in Human Slit-1. This region shows homology to the G-loops of laminin A chains.
Cysteine knots are dimerisation domains defined by the presence of six cysteine residues between which disulphide bridges form. The only absolutely conserved residues are the six cysteines, and spacing between them is highly variable, apart from between cysteines 2 and 3, and 5 and 6: C[x]C[1-3x]GxC[x]C[x]CxC. The glycine between cysteines 2 and 3 is only present in a subset of cysteine knots. Drosophila slit and Human slit-1 both have an extra cysteine after cysteines 5 and 6: this may serve as an intermolecular bond.
Human Slit-1 gene displays the overall structure of the Drosophila gene, and amino acid conservation is found along the entire length of the protein (48% homology at the amino acid sequence excluding the signal sequence; see below). The Human gene has an extra LRR between LRR2 and LRR3 of the first set of LRRs; in the third set, the Human gene has an extra LRR between LRR3 and LRR4. The Human gene has two extra EGF repeats, on either side of the seventh EGF repeat in Drosophila slit.

Isolation of Human slit-1
Searching of the EST database revealed an EST, ab16g10.r1, with homology to the 99aa spacer region of Drosophila slit. This EST was used to probe a Human fetal brain library (Stratagene), and clones for Human slit-1 were isolated.

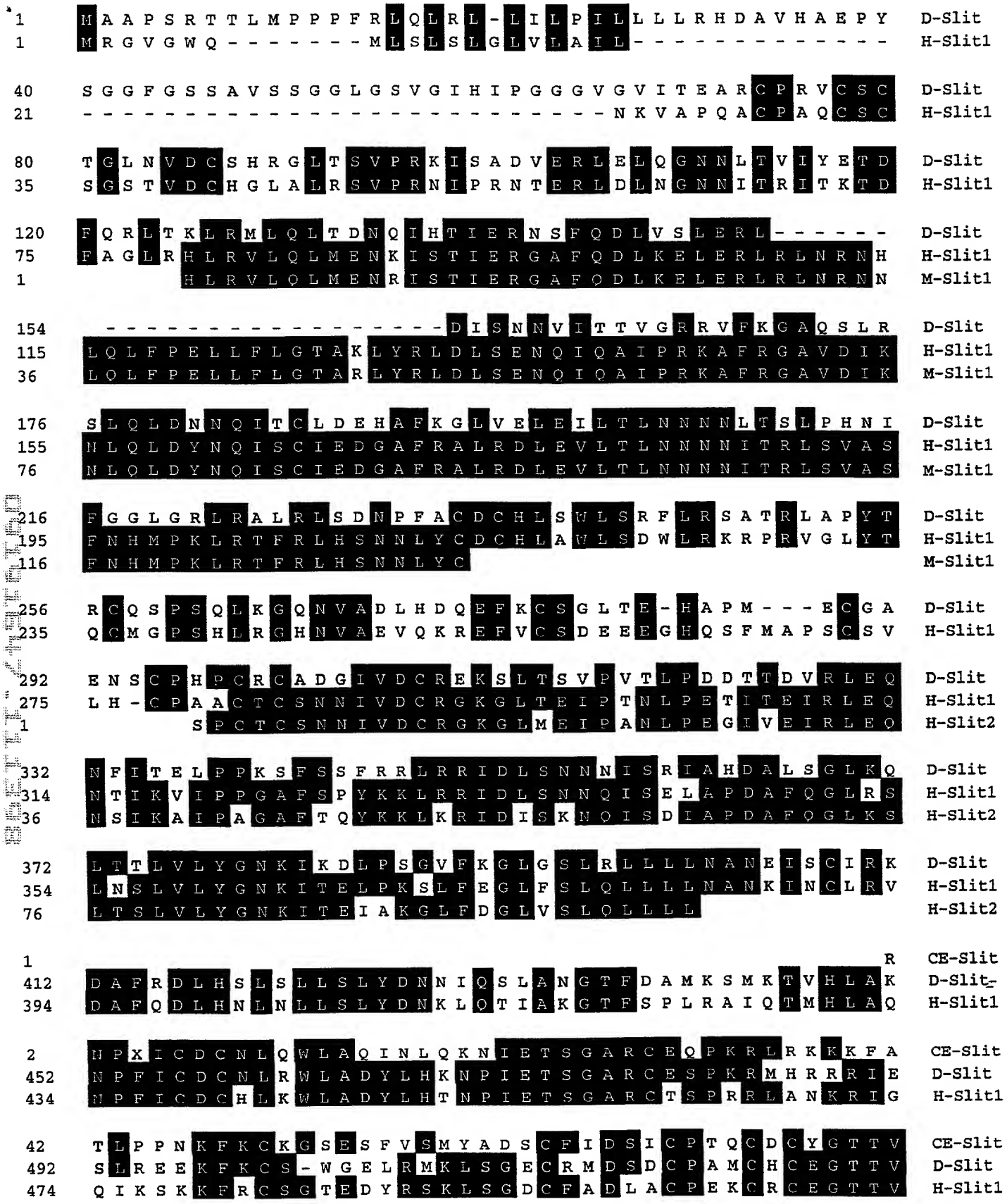
Amino acid identity between Drosophila Slit and Human Slit-1

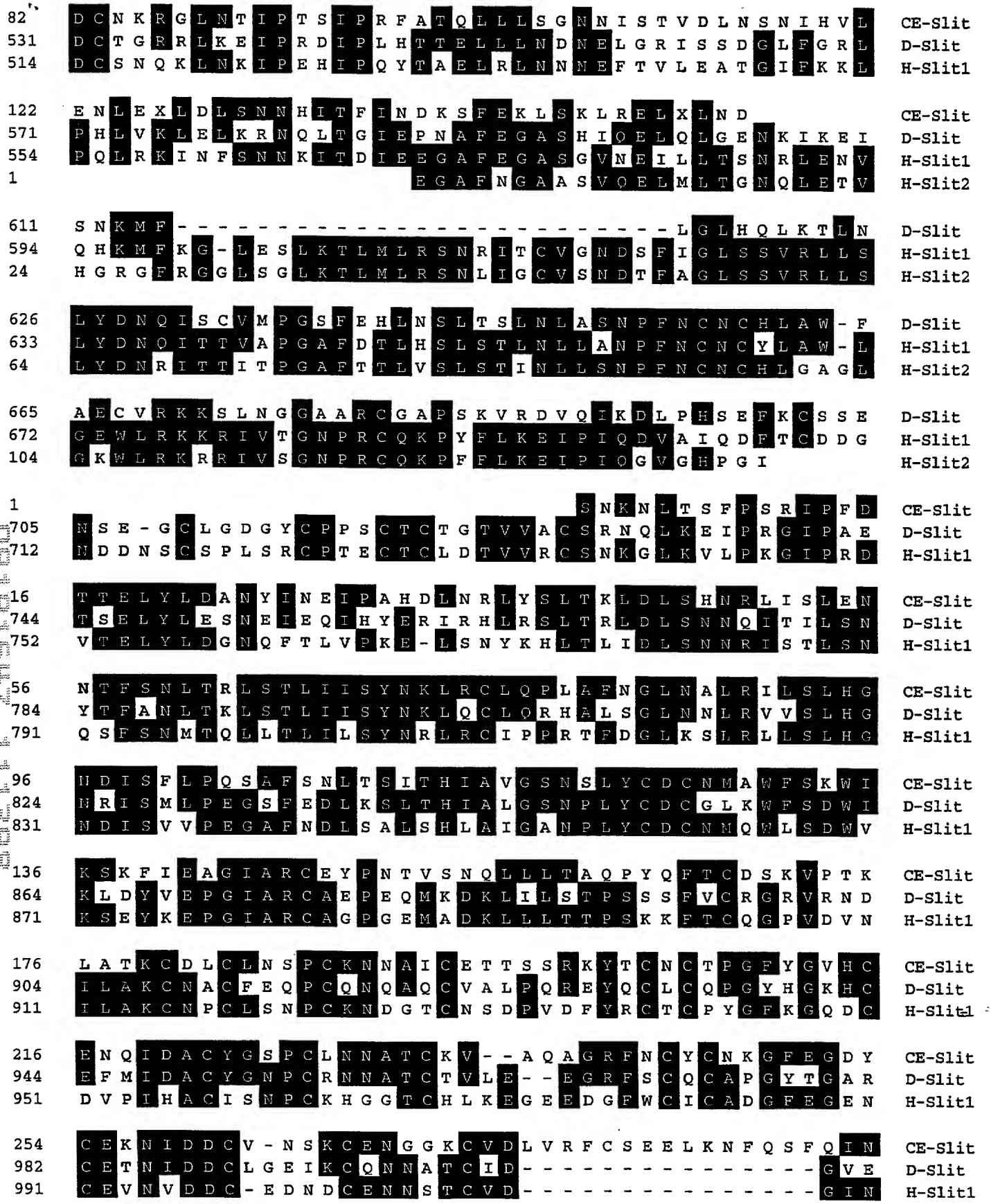
First amino-flanking sequence:	53%	
First set of Leucine Rich Repeats:	52%	(54%, 67%, NA, 38%, 54%, 50%)
First carboxy-flanking sequence:	42%	
Second amino-flanking sequence:	50%	
Second set of Leucine Rich Repeats:	60%	(54%, 58%, 67%, 71%, 50%)
Second carboxy-flanking sequence:	62%	
Third amino-flanking sequence:	56%	
Third set of Leucine Rich Repeats:	49%	(46%, 46%, 42%, NA, 58%)
Third carboxy-flanking sequence:	36%	
Fourth amino-flanking sequence:	53%	
Fourth set of Leucine Rich Repeats:	48%	(25%, 58%, 46%, 63%)
Fourth carboxy-flanking sequence:	63%	
First EGF repeat:	34%	
Second EGF repeat:	46%	
Third EGF repeat:	46%	
Fourth EGF repeat:	35%	
Fifth EGF repeat:	47%	
Spacer:	22%	
Sixth EGF repeat:	40%	
"99aa spacer":	38%	
Seventh EGF repeat:	11%/NA	
Eighth EGF repeat:	44%	
Nineth EGF repeat:	29%/NA	
Cysteine knot motif:	34%	

NA: not applicable due to absence of homologous repeat.
Figures for individual LRRs are shown in brackets.

TABLE 3

Alignment of Slit sequences

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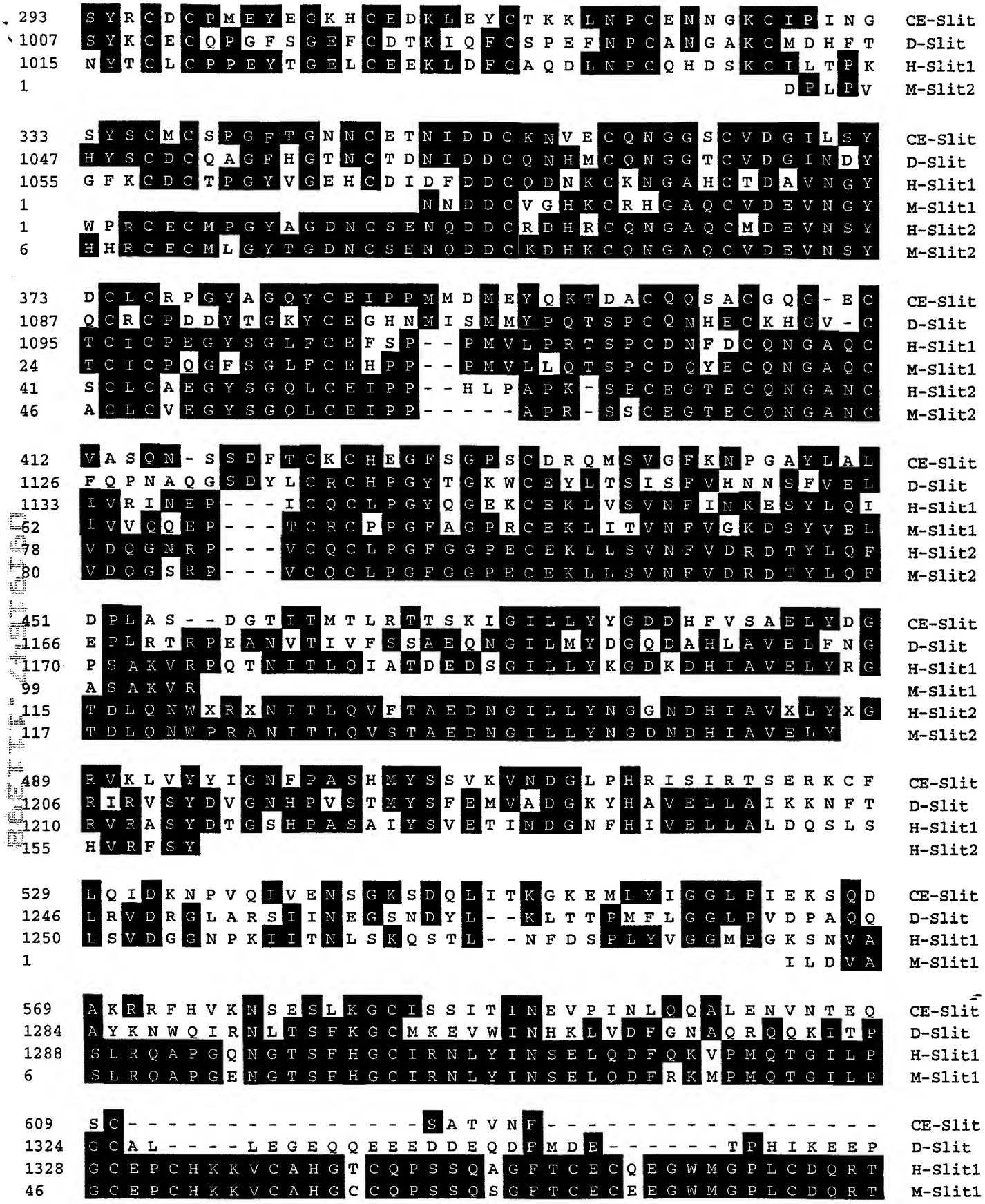
531 DCTGRRLKEIPRDIPLHTTELLLNDNELGRISSDGLFGRL D-Slit
514 DCSNQKLNKIPEHIPQYTAELRLNNNEFTVLEATGIFKKL H-Slit1
122 ENLEXLDLSNNHITFINDKSFEKLSKLRELXLND CE-Slit
571 PHLVKLELKRNQLTGIEPNAFEGASHIQELQLGENKIKEI D-Slit
554 PQLRKINFSNNKITDIEEGAFEGASGVNEILLTSNRLENV H-Slit1
1 EGAFNGAASVQELMLTGNQLETV H-Slit2
611 SNKMF----------L GLHQLKTLN D-Slit
594 QHKMFKG-LESLKTLMLRSNRITCVGNDSFIGLSSVRLLS H-Slit1
24 HGRGFRGGLSGLKTLMLRSNLIGCVSNDTFAGLSSVRLLS H-Slit2
626 LYDNQISCVMPGSFEHLNSLTSLNLASNPFNCNCHLAW-F D-Slit
633 LYDNQITTVAPGAFDTLHSLSTLNLLANPFNCNCYLAW-L H-Slit1
64 LYDNRITTITPGAFTTLVSLSTINLLSNPFNCNCHLGAGL H-Slit2
665 AECVRKKSLNGGAARCGAPSKVRDVQIKDLPHSEFKCSSE D-Slit
672 GEWLRKKRIVTGNPRCQKPYFLKEIPIQDVAIQDFTCDDG H-Slit1
104 GKWLRKRRIVSGNPRCQKPFFLKEIPIQGVGHPGI H-Slit2
1 SNKNLTSFPSRIPFD CE-Slit
705 NSE-GCLGDGYCPPSCTCTGTVVACSRNQLKEIPRGIPAE D-Slit
712 NDDNSCSPLSRCPTECTCLDTVVRCSNKGLKVLPKGIPRD H-Slit1
16 TTELYLDANYINEIPAHDLNRLYSLTKLDLSHNRLISLEN CE-Slit
744 TSELYLESNEIEQIHYERIRHLRSLTRLDLSNNQITILSN D-Slit
752 VTELYLDGNQFTLVPKE-LSNYKHLTLIDLSNNRISTLSN H-Slit1
56 NTFSNLTRLSTLIISYNKLRCLQPLAFNGLNALRILSLHG CE-Slit
784 YTFANLTKLSTLIISYNKLQCLQRHALSGLNNLRVVSLHG D-Slit
791 QSFSNMTQLLTLILSYNRLRCIPPRTFDGLKSLRLLSLHG H-Slit1
96 NDISFLPQSAFSNLTSITHIAVGSNSLYCDCNMAWFSKWI CE-Slit
824 NRISMLPEGSFEDLKSLTHIALGSNPLYCDCGLKWFSDWI D-Slit
831 NDISVVPEGAFNDLSALSHLAIGANPLYCDCNMQWLSDWV H-Slit1
136 KSKFIEAGIARCEYPNTVSNQLLLTAQPYQFTCDSKVPTK CE-Slit
864 KLDYVEPGIARCAEPEQMKDKLILSTPSSSFVCRGRVRND D-Slit
871 KSEYKEPGIARCAGPGEMADKLLLTTPSKKFTCQGPVDVN H-Slit1
176 LATKCDLCLNSPCKNNAICETTSSRKYTCNCTPGFYGVHC CE-Slit
904 ILAKCNACFEQPCQNQAQCVALPQREYQCLCQPGYHGKHC D-Slit
911 ILAKCNPCLSNPCKNDGTCNSDPVDFYRCTCPYGFKGQDC H-Slit1
216 ENQIDACYGSPCLNNATCKV--AQAGRFNCYCNKGFEGDY CE-Slit
944 EFMIDACYGNPCRNNATCTVLE--EGRFSCQCAPGYTGAR D-Slit
951 DVPIHACISNPCKHGGTCHLKEGEEDGFWCICADGFEGEN H-Slit1
254 CEKNIDDCV-NSKCENGGKCVDLVRFCSEELKNFQSFQIN CE-Slit
982 CETNIDDCLGEIKCQNNATCID----------GVE D-Slit
991 CEVNVDDC-EDNDCENNSTCVD----------GIN H-Slit1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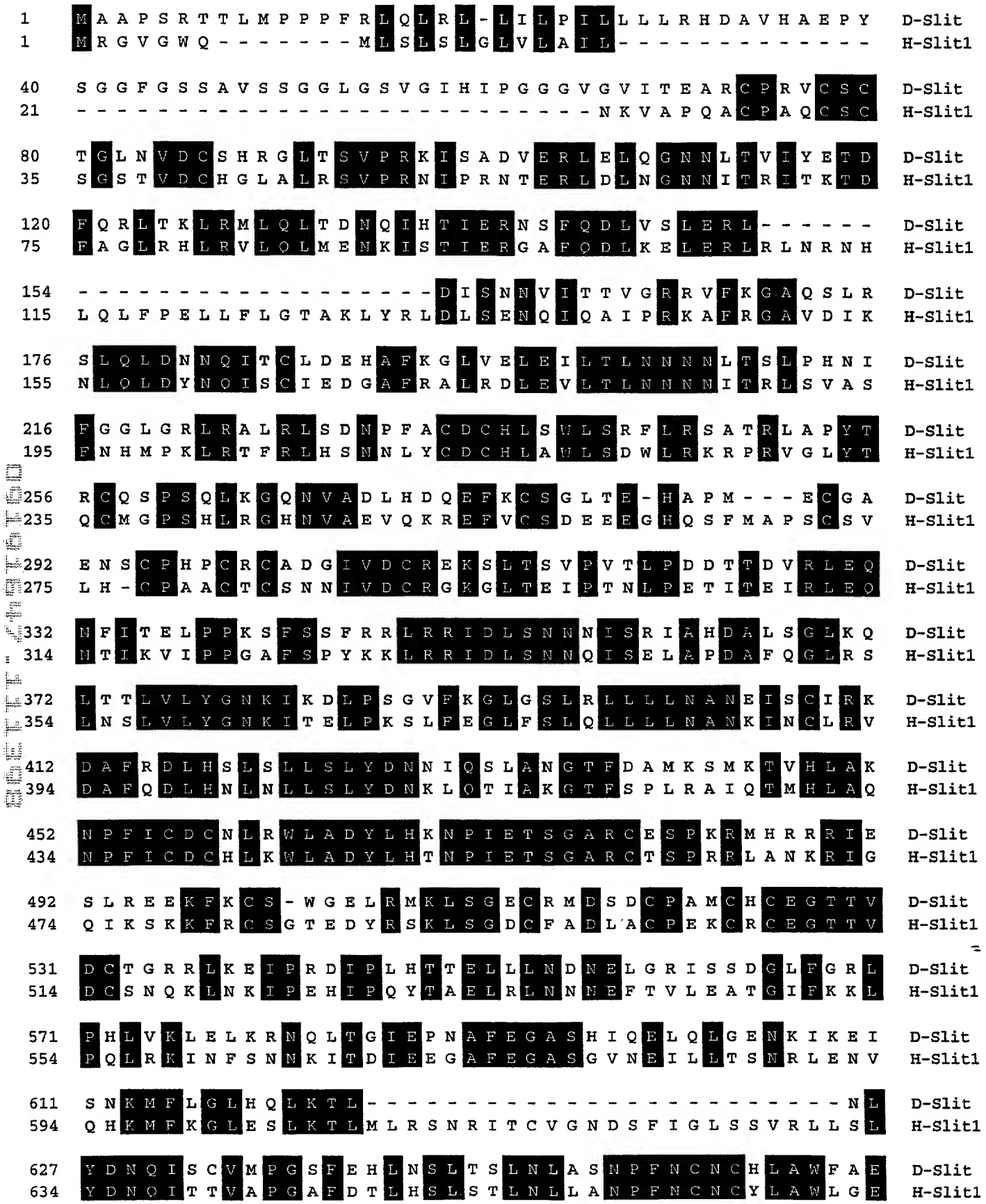
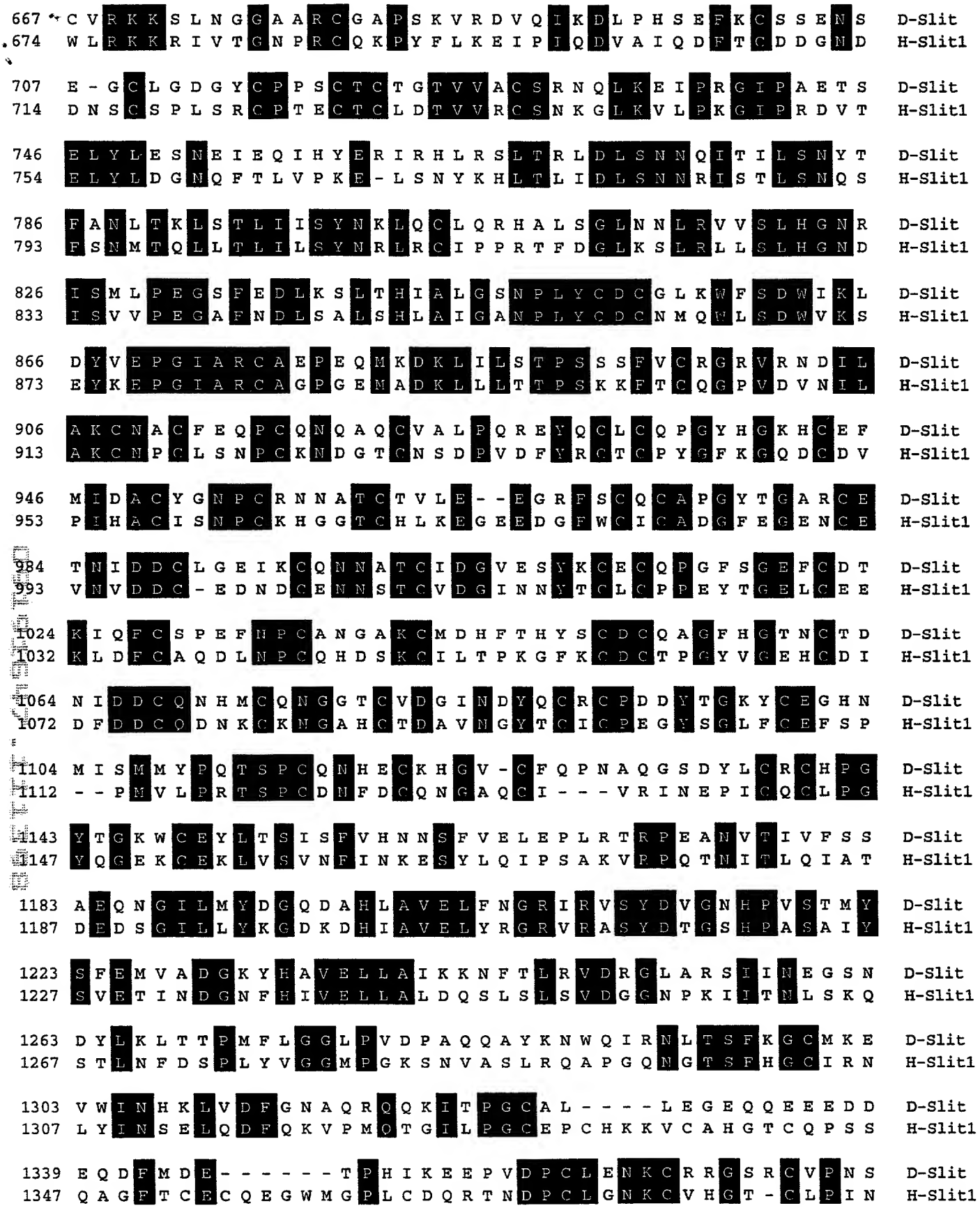
1015 NYTCLCPPEYTGELCEEKLDFCAQDLNPCQHDSKCILTPK H-Slit1
1 DPLPV M-Slit2
333 SYSCMCSPGFTGNNCETNIDDCKNVECQNGGSCVDGILSY CE-Slit
1047 HYSCDCQAGFHGTNCTDNIDDCQNHMCQNGGTCVDGINDY D-Slit
1055 GFKCDCTPGYVGEHCDIDFDDCQDNKCKNGAHCTDAVNGY H-Slit1
1 NNDDCVGHKCRHGAQCVDEVNGY M-Slit1
1 WPRCECMPGYAGDNCSENQDDCRDHRCQNGAQCMDEVNSY H-Slit2
6 HHRCECMLGYTGDNCSENQDDCKDHKCQNGAQCVDEVNSY M-Slit2
373 DCLCRPGYAGQYCEIPPMMDMEYQKTDACQQSACGQG-EC CE-Slit
1087 QCRCPDDYTGKYCEGHNMISMMYPQTSPCQNHECKHGV-C D-Slit
1095 TCICPEGYSGLFCEFSP--PMVLPRTSPCDNFDCQNGAQC H-Slit1
24 TCICPQGFSGLFCEHPP--PMVLLQTSPCDQYECQNGAQC M-Slit1
41 SCLCAEGYSGQLCEIPP--HLPAPK-SPCEGTECQNGANC H-Slit2
46 ACLCVEGYSGQLCEIPP-----APR-SSCEGTECQNGANC M-Slit2
412 VASQN-SSDFTCKCHEGFSGPSCDRQMSVGFKNPGAYLAL CE-Slit
1126 FQPNAQGSDYLCRCHPGYTGKWCEYLTSISFVHNNSFVEL D-Slit
1133 IVRINEP---ICQCLPGYQGEKCEKLVSVNFINKESYLQI H-Slit1
62 IVVQQEP---TCRCPPGFAGPRCEKLITVNFVGKDSYVEL M-Slit1
78 VDQGNRP---VCQCLPGFGGPECEKLLSVNFVDRDTYLQF H-Slit2
80 VDQGSRP---VCQCLPGFGGPECEKLLSVNFVDRDTYLQF M-Slit2
451 DPLAS--DGTITMTLRTTSKIGILLYYGDDHFVSAELYDG CE-Slit
1166 EPLRTRPEANVTIVFSSAEQNGILMYDGQDAHLAVELFNG D-Slit
1170 PSAKVRPQTNITLQIATDEDSGILLYKGDKDHIAVELYRG H-Slit1
99 ASAKVR M-Slit1
115 TDLQNWXRXNITLQVFTAEDNGILLYNGGNDHIAVXLYXG H-Slit2
117 TDLQNWPRANITLQVSTAEDNGILLYNGDNDHIAVELY M-Slit2
489 RVKLVYYIGNFPASHMYSSVKVNDGLPHRISIRTSERKCF CE-Slit
1206 RIRVSYDVGNHPVSTMYSFEMVADGKYHAVELLAIKKNFT D-Slit
1210 RVRASYDTGSHPASAIYSVETINDGNFHIVELLALDQSLS H-Slit1
155 HVRFSY H-Slit2
529 LQIDKNPVQIVENSGKSDQLITKGKEMLYIGGLPIEKSQD CE-Slit
1246 LRVDRGLARSIINEGSNDYL--KLTTPMFLGGLPVDPAQQ D-Slit
1250 LSVDGGNPKIITNLSKQSTL--NFDSPLYVGGMPGKSNVA H-Slit1
1 ILDVA M-Slit1
569 AKRRFHVKNSESLKGCISSITINEVPINLQQALENVNTEQ CE-Slit
1284 AYKNWQIRNLTSFKGCMKEVWINHKLVDFGNAQRQQKITP D-Slit
1288 SLRQAPGQNGTSFHGCIRNLYINSELQDFQKVPMQTGILP H-Slit1
6 SLRQAPGENGTSFHGCIRNLYINSELQDFRKMPMQTGILP M-Slit1
609 SC----------SATVNF---------- CE-Slit
1324 GCAL----LEGEQQEEEDDEQDFMDE------TPHIKEEP D-Slit
1328 GCEPCHKKVCAHGTCQPSSQAGFTCECQEGWMGPLCDQRT H-Slit1
46 GCEPCHKKVCAHGCCQPSSQSGFTCECEEGWMGPLCDQRT M-Slit1


617 ---CAGIDCGNG-KCTNNALSPKGYMCQCDSHFSGEHCDE CE-Slit
1354 VDPCLENKCRRGSRCVPNSNARDGYQCKCKHGQRGRYCDQ D-Slit
1368 NDPCLGNKCVHGT-CLPINAF--SYSCKCLEGHGGVLCDE H-Slit1
86 NDPCLGNKCVHGT-CLPINAF--SYSCKCLEGHGGVLCDE M-Slit1
653 ---------- CE-Slit
1394 GEGSTEP---------- PTVTAAS-- D-Slit
1405 EEDLFNPCQAIKCKHGKCRLSGLGQPYCECSSGYTGDSCD H-Slit1
123 EEDLFNPCQMIKCKHGKCRLSGVGQPYCECNSGFTGDSCD M-Slit1
1 QCHISDQGEPYCLCQPGFSGEHCQ H-Slit2
1 AFKCHHGQCHISDRGEPYCLCQPGFSGHHCE M-Slit2
653 KRIKCDKQKFRRHHIENE----CRSVDRIKIAECNGYCGG CE-Slit
1408 T---CRKEQVREYYTEND----CRSRQPLKYAKCVGGCG- D-Slit
1445 REISCRGERIRDYYQKQQGYAACQTTKKVSRLECRGGCAG H-Slit1
163 REISCRGERIRDYYQKQQGYAACQTTKKVSRLECRGGCAG M-Slit1
25 QENPCLGQVVREVIRRQKGYASCATASKVPIMECRGGC-G H-Slit2
32 QENPCMGEIVREAIRRQKDYASCATASKVPIMECRGGC-G M-Slit2
689 EQNCCTAVKKKQRKVKMICKNGTTKISTVHIIRQCQCEPT CE-Slit
1440 -NQCCAAKIVRRRKVRMVCSNNRKYIKNLDIVRKCGC--T D-Slit
1485 GQ-CCGPLRSKRRKYSFECTDGSSFVDEVEKVVKCGCTR- H-Slit1
203 GQ-CCGPLRSKRRKYSFECTDGSSFVDEVEKVVKCGCAR- M-Slit1
64 PQ-CCQPTRSKRRKYVFQCTDGSSFVEEVERHLECGCLA- H-Slit2
71 TT-CCQPIRSKRRKYVFQCTDGSSFVEEVERHLECGCRA- M-Slit2
729 KSVLSEK CE-Slit
1477 KKCY D-Slit
1523 --CVS H-Slit1
241 --CAS M-Slit1
102 --C-S H-Slit2
109 --C-S M-Slit2

TABLE 4

Alignment of Drosophila Slit and Human Slit-1




667 C V R K K S L N G G A A R C G A P S K V R D V Q I K D L P H S E F K C S S E N S D-Slit
674 W L R K K R I V T G N P R C Q K P Y F L K E I P I Q D V A I Q D F T C D D G N D H-Slit1
707 E - G C L G D G Y C P P S C T C T G T V V A C S R N Q L K E I P R G I P A E T S D-Slit
714 D N S C S P L S R C P T E C T C L D T V V R C S N K G L K V L P K G I P R D V T H-Slit1
746 E L Y L E S N E I E Q I H Y E R I R H L R S L T R L D L S N N Q I T I L S N Y T D-Slit
754 E L Y L D G N Q F T L V P K E - L S N Y K H L T L I D L S N N R I S T L S N Q S H-Slit1
786 F A N L T K L S T L I I S Y N K L Q C L Q R H A L S G L N N L R V V S L H G N R D-Slit
793 F S N M T Q L L T L I L S Y N R L R C I P P R T F D G L K S L R L L S L H G N D H-Slit1
826 I S M L P E G S F E D L K S L T H I A L G S N P L Y C D C G L K W F S D W I K L D-Slit
833 I S V V P E G A F N D L S A L S H L A I G A N P L Y C D C N M Q W L S D W V K S H-Slit1
866 D Y V E P G I A R C A E P E Q M K D K L I L S T P S S S F V C R G R V R N D I L D-Slit
873 E Y K E P G I A R C A G P G E M A D K L L L T T P S K K F T C Q G P V D V N I L H-Slit1
906 A K C N A C F E Q P C Q N Q A Q C V A L P Q R E Y Q C L C Q P G Y H G K H C E F D-Slit
913 A K C N P C L S N P C K N D G T C N S D P V D F Y R C T C P Y G F K G Q D C D V H-Slit1
946 M I D A C Y G N P C R N N A T C T V L E - - E G R F S C Q C A P G Y T G A R C E D-Slit
953 P I H A C I S N P C K H G G T C H L K E G E E D G F W C I C A D G F E G E N C E H-Slit1
984 T N I D D C L G E I K C Q N N A T C I D G V E S Y K C E C Q P G F S G E F C D T D-Slit
993 V N V D D C - E D N D C E N N S T C V D G I N N Y T C L C P P E Y T G E L C E E H-Slit1
1024 K I Q F C S P E F N P C A N G A K C M D H F T H Y S C D C Q A G F H G T N C T D D-Slit
1032 K L D F C A Q D L N P C Q H D S K C I L T P K G F K C D C T P G Y V G E H C D I H-Slit1
1064 N I D D C Q N H M C Q N G G T C V D G I N D Y Q C R C P D D Y T G K Y C E G H N D-Slit
1072 D F D D C Q D N K C K N G A H C T D A V N G Y T C I C P E G Y S G L F C E F S P H-Slit1
1104 M I S M M Y P Q T S P C Q N H E C K H G V - C F Q P N A Q G S D Y L C R C H P G D-Slit
1112 - - P M V L P R T S P C D N F D C Q N G A Q C I - - - V R I N E P I C Q C L P G H-Slit1
1143 Y T G K W C E Y L T S I S F V H N N S F V E L E P L R T R P E A N V T I V F S S D-Slit
1147 Y Q G E K C E K L V S V N F I N K E S Y L Q I P S A K V R P Q T N I T L Q I A T H-Slit1
1183 A E Q N G I L M Y D G Q D A H L A V E L F N G R I R V S Y D V G N H P V S T M Y D-Slit
1187 D E D S G I L L Y K G D K D H I A V E L Y R G R V R A S Y D T G S H P A S A I Y H-Slit1
1223 S F E M V A D G K Y H A V E L L A I K K N F T L R V D R G L A R S I I N E G S N D-Slit
1227 S V E T I N D G N F H I V E L L A L D Q S L S L S V D G G N P K I I T N L S K Q H-Slit1
1263 D Y L K L T T P M F L G G L P V D P A Q Q A Y K N W Q I R N L T S F K G C M K E D-Slit
1267 S T L N F D S P L Y V G G M P G K S N V A S L R Q A P G Q N G T S F H G C I R N H-Slit1
1303 V W I N H K L V D F G N A Q R Q Q K I T P G C A L - - - - L E G E Q Q E E E D D D-Slit
1307 L Y I N S E L Q D F Q K V P M Q T G I L P G C E P C H K K V C A H G T C Q P S S H-Slit1
1339 E Q D F M D E - - - - - - T P H I K E E P V D P C L E N K C R R G S R C V P N S D-Slit
1347 Q A G F T C E C Q E G W M G P L C D Q R T N D P C L G N K C V H G T - C L P I N H-Slit1

```
1373 N A R D G Y Q C K C K H G Q R G R Y C D Q G E G S T E P - - - - - - - - - - - - - - -   D-Slit
1386 A F - - S Y S C K C L E G H G G V L C D E E E D L F N P C Q A I K C K H G K C R   H-Slit1

1401 - - - - - - - - - - - - - - P T V T A A S - - - - - T C R K E Q V R E Y Y T E N D -   D-Slit
1424 L S G L G Q P Y C E C S S G Y T G D S C D R E I S C R G E R I R D Y Y Q K Q Q G   H-Slit1

1423 - - - C R S R Q P L K Y A K C V G G C - G N Q C C A A K I V R R R K V R M V C S   D-Slit
1464 Y A A C Q T T K K V S R L E C R G G C A G G Q C C G P L R S K R R K Y S F E C T   H-Slit1

1459 N N R K Y I K N L D I V R K C G C T K K C Y                                   D-Slit
1504 D G S S F V D E V E K V V K C G C T R - C V S                                   H-Slit1
```

TABLE 5(A)

Hybridisation Probes for regions of Human Slit-1

Hybridisation Probe for the first Leucine rich repeat region

```
TGCCCGGCGCAGTGCTCTTGCTCGGGCAGCACAGTGGACTGTCACGGGCTGGCGCTGCGCAGCGTGCCCAGGAAT    75
ATCCCCCGCAACACCGAGAGACTGGATTTAAATGGAAATAACATCACAAGAATTACGAAGACAGATTTTGCTGGT   150
CTTAGACATCTAAGAGTTCTTCAGCTTATGGAGAATAAGATTAGCACCATTGAAAGAGGAGCATTCCAGGATCTT   225
AAAGAACTAGAGAGACTGCGTTTAAACAGAAATCACCTTCAGCTGTTTCCTGAGTTGCTGTTTCTTGGGACTGCG   300
AAGCTATACAGGCTTGATCTCAGTGAAAACCAAATTCAGGCAATCCCAAGGAAAGCTTTCCGTGGGGCAGTTGAC   375
ATAAAAAATTTGCAACTGGATTACAACCAGATCAGCTGTATTGAAGATGGGGCATTCAGGGCTCTCCGGGACCTG   450
GAAGTGCTCACTCTCAACAATAACAACATTACTAGACTTTCTGTGGCAAGTTTCAACCATATGCCTAAACTTAGG   525
ACTTTTCGACTGCATTCAAACAACCTGTATTGTGACTGCCACCTGGCCTGGCTCTCCGACTGGCTTCGCAAAAGG   600
CCTCGGGTTGGTCTGTACACTCAGTGTATGGGCCCCTCCCACCTGAGAGGCCATAATGTAGCCGAGGTTCAAAAA   675
CGAGAATTTGTCTGCAGTGATGAGGAAGAAGGTCACCAGTCATTTATGGCTCCTTCTTGTAGTGTTTTGCAC      747
```

Hybridisation Probe for the second Leucine rich repeat region

```
TGCCCTGCCGCCTGTACCTGTAGCAACAATATCGTAGACTGTCGTGGGAAAGGTCTCACTGAGATCCCCACAAAT    75
CTTCCAGAGACCATCACAGAAATACGTTTGGAACAGAACACAATCAAAGTCATCCCTCCTGGAGCTTTCTCACCA   150
TATAAAAAGCTTAGACGAATTGACCTGAGCAATAATCAGATCTCTGAACTTGCACCAGATGCTTTCCAAGGACTA   225
CGCTCTCTGAATTCACTTGTCCTCTATGGAAATAAAATCACAGAACTCCCCAAAAGTTTATTTGAAGGACTGTTT   300
TCCTTACAGCTCCTATTATTGAATGCCAACAAGATAAACTGCCTTCGGGTAGATGCTTTTCAGGATCTCCACAAC   375
TTGAACCTTCTCTCCCTATATGACAACAAGCTTCAGACCATCGCCAAGGGGACCTTTTCACCTCTTCGGGCCATT   450
CAAACTATGCATTTGGCCCAGAACCCCTTTATTTGTGACTGCCATCTCAAGTGGCTAGCGGATTATCTCCATACC   525
AACCCGATTGAGACCAGTGGTGCCCGTTGCACCAGCCCCCGCCGCCTGGCAAACAAAAGAATTGGACAGATCAAA   600
AGCAAGAAATTCCGTTGTTCAGGTACAGAAGATTATCGATCAAAATTAAGTGGAGACTGCTTTGCGGATCTGGCT   675
```

Hybridisation Probe for the third Leucine rich repeat region

```
TGCCCTGAAAAGTGTCGCTGTGAAGGAACCACAGTAGATTGCTCTAATCAAAAGCTCAACAAAATCCCGGAGCAC    75
ATTCCCCAGTACACTGCAGAGTTGCGTCTCAATAATAATGAATTTACCGTGTTGGAAGCCACAGGAATCTTTAAG   150
AAACTTCCTCAATTACGTAAAATAAACTTTAGCAACAATAAGATCACAGATATTGAGGAGGGAGCATTTGAAGGA   225
GCATCTGGTGTAAATGAAATACTTCTTACGAGTAATCGTTTGGAAAATGTGCAGCATAAGATGTTCAAGGGATTG   300
GAAAGCCTCAAAACTTTGATGTTGAGAAGCAATCGAATAACCTGTGTGGGGAATGACAGTTTCATAGGACTCAGT   375
TCTGTGCGTTTGCTTTCTTTGTATGATAATCAAATTACTACAGTTGCACCAGGGGCATTTGATACTCTCCATTCT   450
TTATCTACTCTAAACCTCTTGGCCAATCCTTTTAACTGTAACTGCTACCTGGCTTGGTTGGGAGAGTGGCTGAGA   525
AAGAAGAGAATTGTCACGGGAAATCCTAGATGTCAAAAACCATACTTCCTGAAAGAAATACCCATCCAGGATGTG   600
GCCATTCAGGACTTCACTTGTGATGACGGAAATGATGACAATAGTTGCTCCCCACTTTCTCGC               663
```

Hybridisation Probe for the fourth Leucine rich repeat region

```
TGTCCTACTGAATGTACTTGCTTGGATACAGTCGTCCGATGTAGCAACAAGGGTTTGAAGGTCTTGCCGAAAGGT    75
ATTCCAAGAGATGTCACAGAGTTGTATCTGGATGGAAACCAATTTACACTGGTTCCCAAGGAACTCTCCAACTAC   150
AAACATTTAACACTTATAGACTTAAGTAACAACAGAATAAGCACGCTTTCTAATCAGAGCTTCAGCAACATGACC   225
CAGCTCCTCACCTTAATTCTTAGTTACAACCGTCTGAGATGTATTCCTCCTCGCACCTTTGATGGATTAAAGTCT   300
CTTCGATTACTTTCTCTACATGGAAATGACATTTCTGTTGTGCCTGAAGGTGCTTTCAATGATCTTTCTGCATTA   375
TCACATCTAGCAATTGGAGCCAACCCTCTTTACTGTGATTGTAACATGCAGTGGTTATCCGACTGGGTGAAGTCG   450
GAATATAAGGAGCCTGGAATTGCTCGTTGTGCTGGTCCTGGAGAAATGGCAGATAAACTTTTACTCACAACTCCC   525
TCCAAAAAATTTACCTGTCAAGGTCCTGTGGATGTCAATATTCTAGCTAAGTGTAACCCC                  585
```

Hybridisation Probe for EGF repeats one to five

```
TGCCTATCAAATCCGTGTAAAAATGATGGCACATGTAATAGTGATCCAGTTGACTTTTACCGATGCACCTGTCCA    75
TATGGTTTCAAGGGGCAGGACTGTGATGTCCCAATTCATGCCTGCATCAGTAACCCATGTAAACATGGAGGAACT   150
TGCCACTTAAAGGAAGGAGAAGAAGATGGATTCTGGTGTATTTGTGCTGATGGATTTGAAGGAGAAAATTGTGAA   225
GTCAACGTTGATGATTGTGAAGATAATGACTGTGAAAATAATTCTACATGTGTCGATGGCATTAATAACTACACA   300
TGCCTTTGCCCACCTGAGTATACAGGTGAGTTGTGTGAGGAGAAGCTGGACTTCTGTGCCCAGGACCTGAACCCC   375
TGCCAGCACGATTCAAAGTGCATCCTAACTCCAAAGGGATTCAAATGTGACTGCACACCAGGGTACGTAGGTGAA   450
CACTGCGACATCGATTTTGACGACTGCCAAGACAACAAGTGTAAAAACGGAGCCCACTGCACAGATGCAGTGAAC   525
GGCTATACGTGCATATGCCCCGAAGGTTACAGTGGCTTGTTCTGTGAGTTT                           576
```

TABLE 5(B)

Hybridisation Probe for the sixth EGF repeat and preceding spacer region

```
TCTCCACCCATGGTCCTCCCTCGTACCAGCCCCTGTGATAATTTTGATTGTCAGAATGGAGCTCAGTGTATCGTC   75
AGAATAAATGAGCCAATATGTCAGTGTTTGCCTGGCTATCAGGGAGAAAAGTGTGAAAA                  134
```

Hybridisation Probe for the 99aa spacer/G-loop region

```
ATTGGTTAGTGTGAATTTTATAAACAAAGAGTCTTATCTTCAGATTCCTTCAGCCAAGGTTCGGCCTCAGACGAA   75
CATAACACTTCAGATTGCCACAGATGAAGACAGCGGAATCCTCCTGTATAAGGGTGACAAAGACCATATCGCGGT  150
AGAACTCTATCGGGGGCGTGTTCGTGCCAGCTATGACACCGGCTCTCATCCAGCTTCTGCCATTTACAGTGTGGA  225
GACAATCAATGATGGAAACTTCCACATTGTGGAACTACTTGCCTTGGATCAGAGTCTCTCTTTGTCCGTGGATGG  300
TGGGAACCCCAAAATCATCACTAACTTGTCAAAGCAGTCCACTCTGAATTTTGACTCTCCACTCTATGTAGGAGG  375
CATGCCAGGGAAGAGTAACGTGGCATCTCTGCGCCAGGCCCCTGGGCAGAACGGAACCAGCTTCCACGGCTGCAT  450
CCGGAACCTTTACATCAACAGTGAGCTGCAGGACTTCCAGAAGGTGCCGATGCAAACAGGCATTTTGCCTGGCTGT  526
```

Hybridisation Probe for EGF repeats seven to nine

```
GAGCCATGCCACAAGAAGGTGTGTGCCCATGGCACATGCCAGCCCAGCAGCCAGGCAGGCTTCACCTGCGAGTGC   75
CAGGAAGGATGGATGGGGCCCCTCTGTGACCAACGGACCAATGACCCTTGCCTTGGAAATAAATGCGTACATGGC  150
ACCTGCTTGCCCATCAATGCGTTCTCCTACAGCTGTAAGTGCTTGGAGGGCCATGGAGGTGTCCTCTGTGATGAA  225
GAGGAGGATCTGTTTAACCCATGCCAGGCGATCAAGTGCAAGCATGGGAAGTGCAGGCTTTCAGGTCTGGGGCAG  300
CCCTACTGTGAATGCAGCAGTGGATACACGGGGACAGCTGTGATCGAGAAATC                         353
```

Hybridisation Probe for the cysteine knot region

```
TCTTGTCGAGGGGAAAGGATAAGAGATTATTACCAAAAGCAGCAGGGCTATGCTGCTTGCCAAACAACCAAGAAG   75
GTGTCCCGATTAGAGTGCAGAGGTGGGTGTGCAGGAGGGCAGTGCTGTGGACCGCTGAGGAGCAAGCGGCGGAAA  150
TACTCTTTCGAATGCACTGACGGCTCCTCCTTTGTGGACGAGGTTGAGAAAGTGGTGAAGTGCGGCTGTACGAGG  225
TGTGTGTCC                                                                     234
```

TABLE 6

PCR Primers for regions of Human Slit-1

PCR Primers for the first Leucine rich repeat region

Forward: 5' TGCCCGGCGCAGTGCTCTTGCTCGGGCAGC 3'
Reverse: 5' GTGCAAAACACTACAAGAAGGAGCCATAAA 3'

PCR Primers for the second Leucine rich repeat region

Forward: 5' TGCCCTGCCGCCTGTACCTGTAGCAACAAT 3'
Reverse: 5' AGCCAGATCCGCAAAGCAGTCTCCACTTAA 3'

PCR Primers for the third Leucine rich repeat region

Forward: 5' TGCCCTGAAAAGTGTCGCTGTGAAGGAACC 3'
Reverse: 5' GCGAGAAAGTGGGGAGCAACTATTGTCATC 3'

PCR Primers for the fourth Leucine rich repeat region

Forward: 5' TGTCCTACTGAATGTACTTGCTTGGATACA 3'
Reverse: 5' GGGGTTACACTTAGCTAGAATATTGACATC 3'

PCR Primers for EGF repeats one to five

Forward: 5' TGCCTATCAAATCCGTGTAAAAATGATGGC 3'
Reverse: 5' AAACTCACAGAACAAGCCACTGTAACCTTC 3'

PCR Primers for the sixth EGF repeat and preceding spacer region

Forward: 5' TCTCCACCCATGGTCCTCCCTCGTACCAGC 3'
Reverse: 5' TTTTCACACTTTTCTCCCTGATAGCCAGGC 3'

PCR Primers for the 99aa spacer/G-loop region

Forward: 5' ATTGGTTAGTGTGAATTTTATAAACAAAGA 3'
Reverse: 5' ACAGCCAGGCAAAATGCCTGTTTGCATCGG 3'

PCR Primers for EGF repeats seven to nine

Forward: 5' GAGCCATGCCACAAGAAGGTGTGTGCCCAT 3'
Reverse: 5' GATTTCTCGATCACAGCTGTCCCCGTGTAT 3'

PCR Primers for the cysteine knot region

Forward: 5' TCTTGTCGAGGGGAAAGGATAAGAGATTAT 3'
Reverse: 5' GGACACACACCTCGTACAGCCGCACTTCAC 3'